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Physician Assisted Dying (PAD): An Investigation into the Mechanistic Action of Opioid, Benzodiazepine, and Barbiturate Administration as an Alternative Measure to Forgoing Life Sustaining Treatment and Aggressive Palliation.

Jordana Chanel Deighton
Claremont McKenna College

Physician Assisted Dying (PAD): An Investigation into the Mechanistic Action of Opioid, Benzodiazepine, and Barbiturate Administration as an Alternative Measure to Forgoing Life Sustaining Treatment and Aggressive Palliation.

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JORDANA CHANEL DEIGHTON

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Abstract

Whilst modern medicine has increased longevity, the rise in life expectancy has brought about new struggles, namely that of aging and age related disease. Thus, humanity has been presented with a new problem: at what point is death preferable to aggressive life-prolonging treatment in the face of inevitable death? And if so, what right do individuals have to control the circumstances of their death? In the West, traditionally, individuals who seek to end their own lives in the face of terminal illness opt for forgoing artificial hydration and nutrition. Driven by an increase in desire for autonomous dignified death, a new method, physician assisted dying (PAD), has increased in popularity. Due to its novelty, previous studies have concentrated primarily on demographics of PAD or the ethical implications of PAD legislation - fearing possible eugenics-complications for vulnerable societies. Currently, there does not exist a review which contemplates PAD as an option for the critically ill and contrasts it with that of forgoing life sustaining treatment. This includes an investigation into demographics opting for PAD, PAD drug classes and their physiological action in inducing death, and an analysis of case studies . PAD offers a succinct and decisive opportunity to end one's life, without the fear of enduring the extended dying process and unforeseen psychological and physiological changes of forgoing treatment. Contrary to what one might assume, PAD in fact promotes thorough decision-making and preparedness for death through encouraged patient autonomy. These encouraging prospects suggest that patients considering death should be offered multiple options in order to make an informed decision as to which plan is most appropriate for their circumstances. I propose that awareness and acceptance of PAD as a form of humane end of life care should be exemplified. In

this way, palliative care givers can continue to support patients in attaining a life which is consistent with patient goals and values.

Introduction

Physician assisted dying (PAD) and euthanasia is a practice where physicians provide, at the request of the patient, a lethal dose of self-administered medication which the patient takes with the intention of ending their life. Historically, it is thought PAD exists outside the realm of medical intervention, with many believing the act goes against the philosophical ideology of medicine, due to its perceived opposition to the Hippocratic oath (insert research on physician perception). It is the case however, that PAD transcends time. Voluntary euthanasia and phsycian assisted suicide was commonly practiced in ancient Greece to spare people of high social rank from prolongued suffering (Ardelt 2003). In recent years, we have observed the unraveling of this oppositional ideology and an increase in the occurrence of PAD as a palliative treatment globally. This is in part, due to the rise in support of patient autonomy for life ending procedures for those individuals suffering from terminal illness or incurable psychiatric disorders (resource stat on percentage). The perception around the desire to die is evolving from one of unspoken-ness to one which deals with the ideas of dignity, humanity, morality, and beliefs in the face of death. The realm of PAD is one littered with many ethical, medical, and legal constraints. As such, it is an important time to reanalyze and refresh our understanding of the current methods of PAD, including the biological actions of commonly used drugs from their molecular, cellular, and systems level effects. I will first define the process, establish the demographic

characteristics of PAD-requests, and outline the process of dying for alternative methods. This will serve as a preliminary lens, through which we can comprehend relevant background information and generate a comparison to effectively evaluate PAD. PAD is predominantly accomplished with two classes of drugs, the GABAergic agonists, barbiturates and benzodiazepines, and opiates. I intend to illuminate PAD as an alternative measure of forgoing life sustaining treatment (artificial nutrition and hydration) for individuals suffering with terminal illness. This analysis should minimize concerns regarding the process and reinstate PAD as an intimate, ethically humane response to human suffering.

Physician assisted dying (PAD)

What is PAD?

Before we begin our investigation into the cellular-molecular mechanisms of relevant drugs, it is important that we outline what we mean by "euthanasia" and "Physician-Assisted Dying". *Euthanasia* is the clinician administration of lethal agent with the intent of relieving a patients untreatable suffering or pain, whereby the agent of death is the clinician (Post 2015). *Aid in Dying* is the clinician facilitation of a patient's death by providing the means and information that enable the patient to perform an act that results in death – the agent of death is the patient (Post, 2015). As I have mentioned previously, the debate regarding PAD has arisen as a result of the tensions between valid concerns about the autonomy of an individual to prevent slow, painful, suffering, undignified prolonged dying and society's obligation to protect its most vulnerable (Ardelt 2003). Although generally, voluntary passive euthanasia is a legal and accepted practice, the prominence of physician assisted dying and its legality is variable. In the

US, Oregon, Colorado, the District of Columbia, Hawaii, Maine, New Jersey, New Mexico, Vermont, and Washington have legalized the process, with Montana and California extending the option to patients via a court ruling. In the Netherlands and Belgium, PAD is legal under certain conditions, and in Switzerland an individual will only face prosecution if they initiate death for selfish reasons (Ardelt 2003). Due to the relatively recent legislation of PAD in the US, the majority of existing studies on the topic focus on the regions of western Europe due to the extensive documentation and studies conducted on the matter (Ardelt 2003). In Oregon, since the enactment of the Oregon Death with Dignity Act in 1997, 1-2 individuals out of every 1000 who have died in Oregon opted for PAD (Ganzini 2009).

Demographics

At the core of the public and professional debate regarding this topic, lies the goal of respecting individual self-determination, even if this means extending this autonomy to meet requests for death. The motivation underlying this phenomenon lies in the desire for patients to possess the ability of freeing themselves from physical, or mental induced suffering (AMA, year). This is reinforced by data outlining the demographics and conditions of individuals who request PAD (**Table 2**). Indeed, 93.4% of individuals who choose PAD are terminally ill and of these individuals, 87% have late-stage cancer (**Table 2**). Quality of life for these surveyed individuals was significantly lower than the average population with 96% reporting physical suffering, 66.5% with mental suffering, and 64.7% with both. In addition, all patients who reported that they were not physically suffering, reported psychological suffering (Smets, 2010).

Furthermore 42% of those who requested death felt as though they had lost their dignity in their latter moments of life. Those who were not suffering with terminal illness (6%) reported greater psychological suffering (89.7%) than terminally ill patients (66.5%) (Smets 2010). These individuals also reported greater loss of dignity and greater dependencies than terminal patients, with 47.5% and 57.5% respectively[BD4] reporting these qualities. This highlights the necessity to consider all aspects of quality of life including those which do not manifest in observable physiological detriments. Although these non-terminal individuals are not at risk of death as terminal patients, they too possess the autonomy to decide the consequences of their suffering. For the sake of my investigation, as terminal patients appear most frequently, I will be exclusively considering those individuals who are terminally ill.

Characteristic	All Reported Cases of Euthanasia N = 1917	Terminally Ill Patients N = 1790 (93.4)	Nonterminally Ill Patients N = 126 (6.6)	*P*
Diagnosis				<0.001†
Cancer	82.5	87.6	9.2	
Other than cancer	17.5	12.4	90.8	
Progressive neuromuscular disease	7.3	5.1	37.9	
Cardiovascular disease	2.4	2.0	8.9	
Non-malignant	1.9	1.7	4.0	
Pulmonary disease				
Nonprogressive neuromuscular disease	1.0	0.2	13.7	
AIDS	0.4	0.3	0.8	
Other	4.5‡	3.1	25.0	
Reported suffering§				
Physical suffering	95.6	96.0	89.7	0.001
Psychological suffering	68.0	66.5	89.7	<0.001
Physical and psychological suffering	64.7	63.7	79.4	0.001
Nature of reported physical suffering¶				
Pain	53.6	54.7	41	0.101
Cachexia, exhaustion	32.5	33.6	20.5	0.095
Dysphagia, vomiting, bowel obstruction	28.3	29.0	20.5	0.260
Dyspnoea	22.9	23.7	12.8	0.119
Severe wounds	5.4	5.9	0	0.119
Hemorrhage	2.8	3.1	0	0.269
Other	25.3	23.5	46.3	0.001
Nature of reported psychological suffering¶				
Loss of dignity/despair	42.5	42.0	47.5	0.503
Dependency	26.1	23.3	57.5	<0.001
Other	1.7	1.4	5.6	0.028

Data presented are column percentages; *P* values calculated with Fisher exact test. Percentages may not always amount to 100% because of rounding.

*The euthanasia law makes a distinction between patients who are expected to die within the near future and patients who are not expected to die within the near future. Within the near future is defined by the Federal Control and Evaluation Committee as dying within the next few months. Patients who were not expected to die within the near future were patients who were not expected to die within the next few months. It is the attending physician who evaluates the terminality of the patient's disease.

†*P* value for cancer versus other than cancer.

‡Including, among others, 18 cases of neuropsychiatric disease: depression (n = 5), Huntington disease (n = 5), Alzheimer disease (n = 5), Creutzfeldt-Jacob disease (n = 1), vascular dementia (n = 1), psychosis (n = 1).[9–11]

§For 22 patients no suffering was reported. Seven of these patients were comatose; for the remaining patients, information on the variables of suffering was missing. We could not determine whether the Committee had contacted the physicians for further information.

¶Data for nature of physical and psychological suffering are only available for 499 of the reported euthanasia cases.

Table 2. Clinical characteristics according to whether the patient was terminally ill or not terminally ill at the time of euthanasia (Smets 2010)

In order to determine primary motivators for opting for PAD, 56 Oregonians who either requested PAD or contacted an agency regarding PAD completed a survey indicating the importance of 29 categories in their decision making (Ganzini 2009). The most important

reasons for requesting PAD were determined to be those which relate to controlling the circumstances of one's death (Ganzini 2009). These included a loss of independence, concerns about future pain, poor quality of life, and an inability to care for ones' self. It was determined that proxies ranked factors like depression, poor social support, uncontrolled physical symptoms as less important than the desire for control over circumstances of death (Gazini 2009).

Over time, the demographics of individuals who have opted for euthanasia has shifted (**Table 3**). For relevance to today's climate I will refer to the latest column under 2013. The mean number of patients are over the age of 80, and 81% of the entire population is over 65, suggesting this method of death is most popular for those in the aging population. There is however, no significant difference between the sex of people opting for the procedure, with men leading the gap by only 2%. Given that the predominant age group are those over 65 suffering from terminal illness, I will contrast the methods of PAD with options which would be most available for individuals of their circumstance - that is, forgoing artificial nutrition and hydration in a hospital setting rather than other illicit methods one might investigate like inorganic bath salts, CO_2 poisoning, or violent methods of suicide.

Characteristics	1998	2007	2013
Total number of deaths (unweighted)	1925	3623	3751
Number of euthanasia deaths (unweighted)[a]	25	142	349
Percentage of all euthanasia deaths (weighted)	1.2	2.0	4.6
Sex			
Male	40.4	61.3	51.0
Female	59.6	38.7	49.0
Age (yrs)			
18–64	35.8	37.0	18.9
65–79	29.0	42.6	37.8
80 or older	35.2	20.4	43.2
Cause of death			
Cardiovascular disease[b]	14.1	3.8	14.3
Malignancies	46.1	80.2	57.4
Respiratory disease	11.1	4.7	4.1
Disease of the nervous system	7.6	7.2	7.4
Other disease	21.1	4.0	16.9
Place of death			
At home	48.4	43.1	41.8
Hospital	43.1	51.3	42.5
Care home	8.6	5.6	15.6
Other	0.0	0.0	0.1

Weighted row percentages.
[a]Numbers include three cases of physician-assisted suicide in 1998, five cases in 2007, and six cases in 2013. Physician-assisted suicide, that is when patients administer the lethal drugs themselves, is treated as a form of euthanasia by the Belgian Euthanasia Review Committee, although it is not mentioned in the euthanasia law.
[b]Includes cerebrovascular disease.

Table 3. (a) Characteristics of death by euthanasia (Sigrid 2018)

Process of dying

In order to make a decision regarding whether PAD is a necessity, and actually fulfills its intended goal, we must contrast the experiences of administration with that of its alternative: death by dehydration and starvation. I have previously outlined my target group as terminally ill cancer patients, as these are the patients most commonly opting for PAD, therefore this is the most likely alternative cause of death as most terminally ill patients have reduced oral intake in

the last days of life. The reasons for this reduction are: those related to cancer treatment, such as dysphagia, anorexia, nausea, and vomiting, or mechanical problems like physical obstruction due to malignancies (Raijmakers 2011). These patients therefore require medical treatment for dehydration and malnutrition in the form of artificial nutrition and artificial hydration. Professional caregivers working in palliative care were surveyed to be skeptical about the benefits of artificial nutrition and hydration, reporting that they do not believe that the process contributes to the alleviation of symptoms (Raijmakers 2011). This suggests that the benefits of providing artificial hydration and nutrition are not significant enough to overcome the negative experience of living as a terminal cancer patient.

Patients considered competent then, can decide to opt to forgo terminal dehydration and malnutrition by choosing to refrain from eating or drinking. Normally, this method is accompanied by palliative care measures, such as pain management, which allow them to hasten death and escape suffering. When a person voluntarily stops eating, death occurs in 1 to three weeks (**Table 4**; Lachman 2015). A characteristic of this method is that individuals have the opportunity to change their minds. For those voluntarily giving up food and water, artificial nutrition and hydration can always be reinstated - the action offers the primarily peace of mind that the patient is in control of their circumstance, and therefore always has a pathway out of their suffering. Some individuals may prefer this option as it allows for a reversible prolonged dying phase which gives the patient the opportunity to mourn, reflect, and manage relationships (Janson 2015).

Issues	VSED	PAS
Method	Stopping eating and drinking	Killing oneself by ingesting prescribed lethal medication
Assistance provided	Support of caregivers to manage palliative care needs (e.g., mouth care, turning, etc.)	Supplying the medical means of causing death
Time	1-3 weeks	Within minutes-hours
Outcome	Death through terminal dehydration	Death through overdose of barbiturates

Table 4. comparison of voluntary stopping of eating (VSED) with physician assisted dying (PAD) with respect to key differences. (Lachman 2015)

Consequences of reduction of fluids results in less urine, less gastrointestinal secretions, less pulmonary and pharyngeal secretions, and edema (Lachlan 2015). In the end stages of terminal hydration, the combination of physiological imbalances and pain management can make the process feel pain free. For example, physiological imbalances can cause analgesia, the inability to feel pain, through: acidosis, the increase in acidity of blood plasma; hypernatremia, the increase of sodium in blood; hypocalcemia, the reduction of calcium in the blood, and cerebral anoxia (Critchlow 2002). As malnutrition and nutrient deprivation begins to onset, ketone production is upregulated which results in a partial loss of sensation - some ketones have shown in rats to induce anesthesia and promote the production of natural opiates (Critchlow 2002). There are however negative problems associated with forgoing life sustaining hydration and nutrition, namely that of lethargy, coma, confusion, pulmonary embolism, headaches, nausea, committing, muscle cramps, thirst, and uncomfortable dry mouth (Lachman 2015). According to mean scores on the discomfort scale among Alzheimer;s patients after forgoing hydration and nutrition, the greatest level of discomfort for all lifespans was on day zero, the

day (**Figure 1**). For patients dying within 5 days, such patients reported a decrease in discomfort in their last moments, whereas survival past day 5, was associated with an increase in discomfort, though never as bad as day 0 (Pasman 2005).

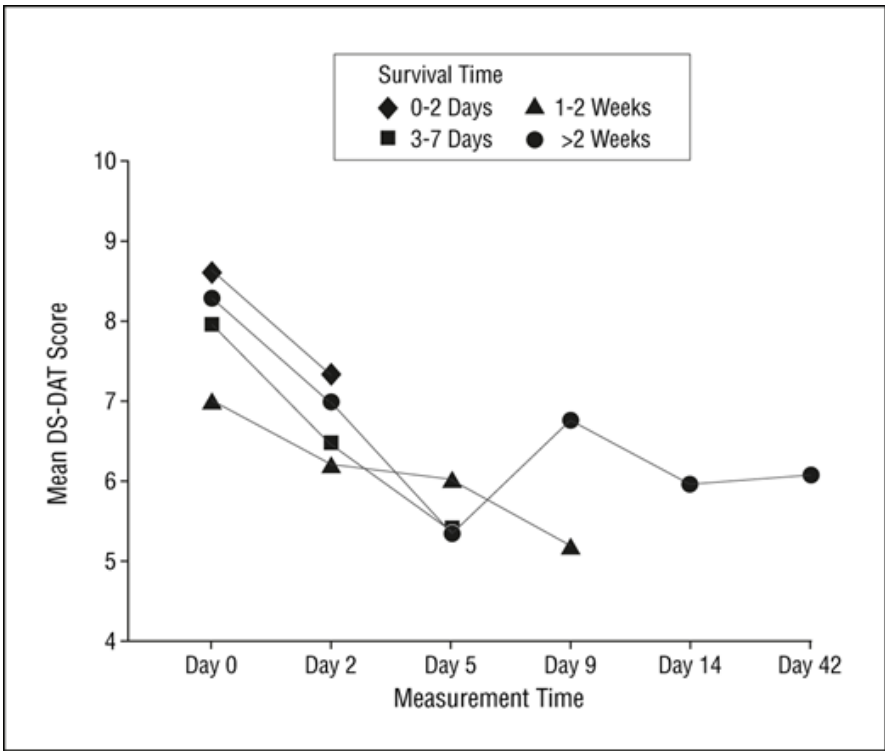

Figure 1. Mean scores on the Discomfort Scale–Dementia of Alzheimer Type according to survival.0-2 days, n=49-11. 2-7 days n=55-16. 1-2 weeks n=28-1. >2 weeks n=47-29 (Pasman 2005).

*Case s*tudy

The following describes the case study of a woman forgoing artificial nutrition and hydration in order to speed up the dying process, it outlines the physiological

changes occurring prior to death. Day 0 represents the day in which artificial nutrition by gastric tube and hydration was withdrawn, and phenytoin, an anticonvulsant, was stopped. The nutritional status of the woman was evaluated as normal before the withdrawal of artificial sustenance.

On day 0, three hours after forgoing treatment, the patient exhibited drooling, sweating, noisy breathing ,and a decrease in diuresis.On day 1, the drooling persisted, diuresis was significantly reduced, body temperature increased moderately, sweating disappeared, and the discomfort scale for dementia - Alzheimer's type (DS-DAT) revealed an absence of discomfort.On Day 2, drooling persisted with dense mucosal secretions, smelly urine was produced, hypotensive signs were observed, noisy breathing reoccurred, and body temperature rose significantly. On day 3, drooling ceased, and the patient was recorded to be anemic and hyperthermic. The DS-DAT scores showed again, a complete absence of discomfort. Late in the afternoon, severe hypotension and bradycardia with dyspnoea appeared, immediately followed by death due to cardiorespiratory arrest.

(Moreschi 2013)

As it is important to contrast this alternative with the consequences and methods of PAD, the rest of this study will therefore concern exclusively the mechanisms of action of PAD drugs in overdose leading to death

Drugs utilized for PAD

Epidemiological studies and case reports of PAD, predominantly Dutch studies, have reported that many drugs have been used to varying effectiveness in bringing about lethal outcomes. One particular study, conducted by G van der Wal, determined that whilst over 40 different drugs have been used, 30% of patients were administered a single drug (a benzodiazepine or opioid), and 57% were administered a cocktail of drugs, which most often consisted of a benzodiazepine and barbiturate along with a neuromuscular relaxant (Willems 1999). In another study from the Netherlands, morphine was used 25% of the time, and the benzodiazepine-barbiturate-neuromuscular relaxant cocktail was used in 50% of cases (Willems 1999; **Table 5**). There are, however, some discrepancies. For example, in an Oregan[BD2] study, all but one patient was administered barbiturates alone and a study conducted in Amsterdam found that all patients were administered barbiturate-benzodiazepine cocktails alone (38%). While the predominant drug of choice varies across countries and demographics, the two major methods of administration are barbiturate-benzodiazepine-neuromuscular relaxant combinations, and opiates.

Drugs	Euthanasia[3,10,15]		PAS[3,10,12,15,16]	
	frequency (%)	effectiveness	frequency (%)	effectiveness
Opioids only	17-25	Variable duration[3,10]		
Oral barbiturates only			>80	Mostly <1h, but variable[3,12]
Barbiturates/benzodiazepines + neuromuscular relaxant[3]	31-50	Mostly <1h		
Benzodiazepines only			<12	Very variable[24]
Insulin	<5	Very variable, adverse effects[17]		
Potassium chloride	<5	Very variable, adverse effects[23]		
Propofol	<5	Unclear[19]		

Table 5. Frequency of use and effectiveness of drugs used in different types of physician assisted death (Willems 1999)

The time elapsed before the onset of death varies by mechanism (drug administration or forgoing sustenance). A 1995 study in Germany investigated the time elapsed between administration of PAD drugs and death and found that 85% of patients died within one hour of euthanasia onset and 96% died within one day. In addition, death occurred on average 5 minutes after intravenous injection and 21 minutes after oral administration (Willems 1999). Similarly, a study in Oregon USA in 1999 found the median death time to be 26 minutes, ranging between 15 minutes to 11.5 hours (Chin 1999). Although PAD is associated with an abrupt death, studies of patients in Oregon showed that approximately half of patients prescribed life ending barbiturates actually used the lethal dose of medication (Lachlan 2015). As such, considering the type and timing of the methods of PAD, it is of paramount importance to understand the mechanisms and psychological effects of these drugs and their target action sites.

GABA

GABA is the most common neurotransmitter in the central nervous system (CNS) and is found in the highest concentrations in the cortex and limbic system. It functions as the primary inhibitory neurotransmitter in the CNS and its action reduces the excitability of neurons (Griffin 2013, Allen 2021). GABAergic neurons form an extensive neural network projecting broadly within regions of the hippocampus, thalamus, basal ganglia, hypothalamus, and brainstem. GABA has also been found in a variety of tissues outside the brain and spinal cord including the optical nerve where GABA modulates axonal excitability; the heart where GABA modulates sinus node; the kidneys, where GABA modulates calcium and/or potassium channels; the adrenal glands, where it is involved in catecholamine release; the stomach, where GABA is involved in acid secretion, is mucoprotective, and stimulates gastrin release; and the lungs, where GABA modulates airway tonus and secretion (Watanabe 2002). GABA exerts its effects via two types of receptors: Ionotropic $GABA_A$ receptors, responsible for mediating fast ionic responses; and metabotropic G-protein-coupled $GABA_B$ receptors, which elicit slower, prolonged effects (Bowrey, 2002).

Synthesis

GABA is produced via the GABAergic shunt cycle, a three-stage process which involves three enzymes; glutamate decarboxylase (GAD), GABA transaminase (GABA-T), and succinate semialdehyde (SSADH; Vargas 2018). Glucose, derived from the Krebs cycle, is used to

synthesize glutamate. Glutamate undergoes decarboxylation in catalysis by GAD to produce GABA (**Figure 2**). The resultant GABA is then stored in vesicles which travel to the presynaptic terminals where it is released in a calcium-dependent mechanism upon depolarization at the synaptic terminal in response to pre-synaptic neuronal activation (Vargas 2018).

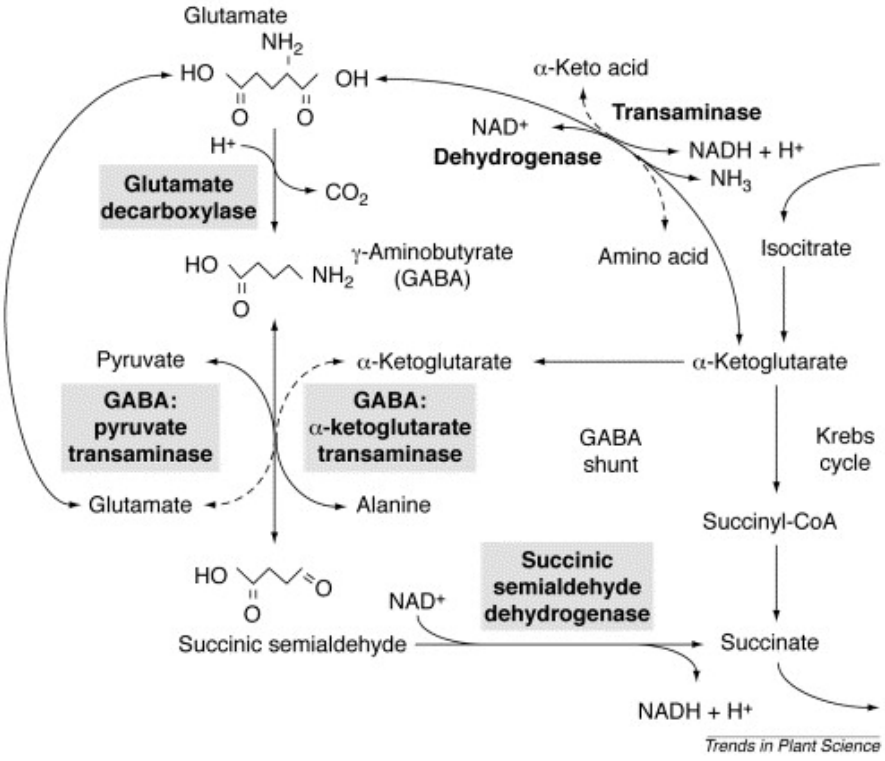

Figure 2: Major metabolic pathway of GABA. GAD, glutamate decarboxylase; GABA-T , γ-amino-butyrate transaminase; SSADH, succinic semialdehyde dehydrogenase.

$GABA_A$

$GABA_A$ receptors are characterized as ligand-gated ion channels, or ionotropic receptors, that bind to GABA released into the synaptic cleft (Allen, 2021). Occupation of the agonist GABA in binding sites of the α/β subunit interface results in a conformational change in protein structure which locks the agonist into place (Wagner, 2001). From here the mechanism is as follows: upon the GABA binding to receptors a series of confirmation changes then occurs resulting in the opening of the gated ion channel. As the pore domain of the channel is positively charged, negatively charged chloride ions move into post-synaptic cells, hyperpolarize such cells, and thus prevent excitation (Sigel 2012). In addition to GABA binding sites, these receptors also contain binding sites for other natural, and manufactured substances - the most relevant for PAD being benzodiazepines and barbiturates (Rayes 2012, **Figure 3**).

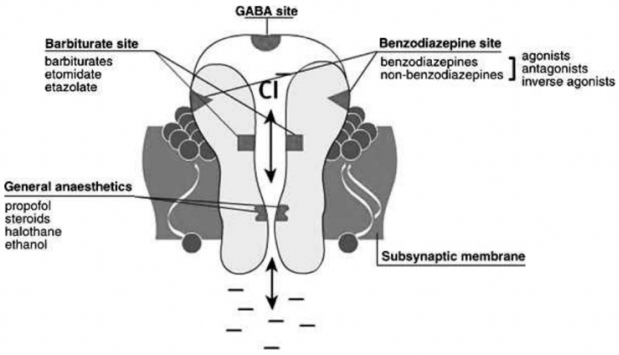

Figure 3. Gamma amino butyric acid receptor with target sites.

As $GABA_A$ receptors are composed of α, β, γ, and δ subunits in a heteropentameric structure, each of which moderates a different pharmacological effect and influences potency and efficacy, GABA can mediate many functions (Rayes 2012, Wu 2015). Research has been conducted to investigate the roles of each subunit by employing the use of in vivo point mutations. Such

experiments found α-1-GABAA receptors mediate sedation, anterograde amnesia, and partially mediate anticonvulsant activity, while α-2-GABAA receptors were found to mediate anxiolysis (Riss 2008). Differences in whether these subunits are extrasynaptic or synaptic also contributes to their qualities as receptors; extrasynaptic $GABA_A$ subunits such as α4, α5, and α6 are characterized a high affinity GABA receptors implicated in inhibition, whereas synaptic $GABA_A$ subunits such as α1 and γ2 are involved in fast, phasis inhibition (Wu 2015). Furthermore, each $GABA_A$ subunit has a unique distribution pattern throughout the brain: (1) α1, β2, β3,γ2 are widely distributed and follow the same distribution pattern; (2) α1 subunit is expressed where α2 is absent; (3) α2, α3, α5, δ are limited to certain brain regions; (4) α3 is expressed only where α1 is expressed at low levels; (5) α4 is abundant in the hippocampus; (6) α5 is expressed in regions correlated to memory function; (7) and α6 is expressed exclusively in cerebellar granule cells (Watanabe 2002) (**Figure 4)**.

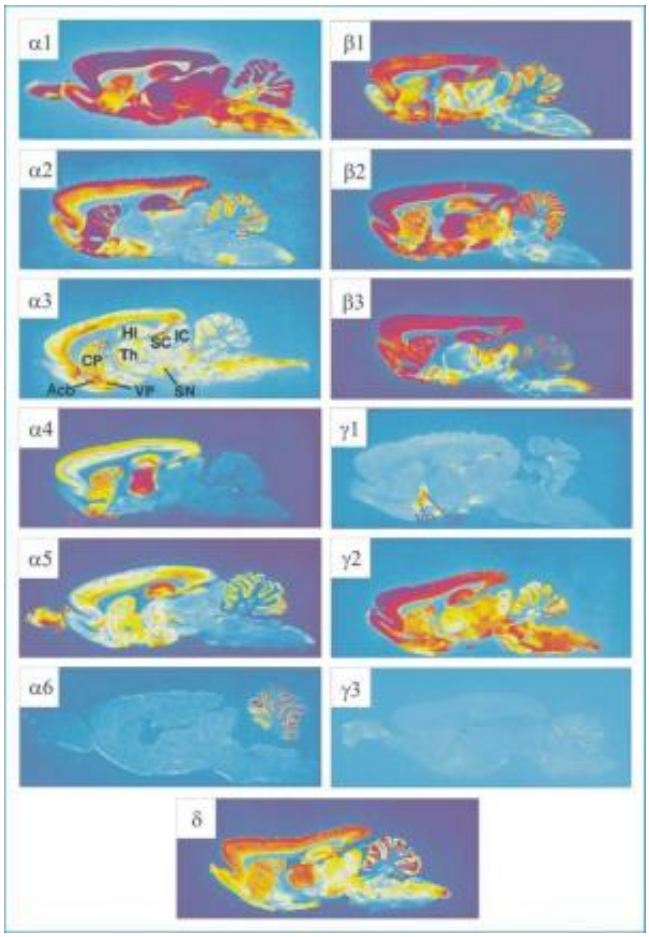

Figure 4. Regional distribution of GABA-A receptor subunits (Pirker 200)

$GABA_B$

$GABA_B$ functions both pre-synaptically and post-synaptically; inhibiting the release of neurotransmitters in the former and leading to hyperpolarization in the latter (Shaye, 2020). $GABA_B$ functions as a constitutie heterodimer, a protein composed of two polypeptide chains differing in composition in the roder, number, or kind of amino acid residue; these distinct subunits are GB1 and GB2. Each subunit consists of the same three domains; extracellular venus fly trap module (or VFT named due to its physical resemblance to the plant), seven helix

transmembrane region, and cytoplasmic tail. Together, these structures influence the basis of ligand recognition, receptor activation, and heterodimerization (Frangaj and Fan, 2018).

The signaling pathway of the $GABA_B$ receptor involves one of the three following effector proteins: voltage gated calcium ion channels, G protein activated potassium channels, or adenylyl cyclase -the outcome of such a pathway results in the blockage of neurotransmitter release and the hyperpolarization of neurons (Bowery, 2002). $GABA_B$ displays both allosteric, and orthosteric ligand recognition. In orthosteric recognition, the ligand-binding subunit $GABA_{B1}$ has its active and closed conformation stabilized by an agonist bound to the VFT; antagonist binding limits the subunit to an inactive closed formation (**Figure 5**; Frangaj and Fan, 2018). Allosteric recognition is determined by three modifiers: positive allosteric modulators which increase the efficacy of orthosteric ligands; and negative allosteric modulators which decrease efficacy; silent, or neutral, modifiers which compete with the former modifiers for allosteric sites but do not play a role in altering receptor activity (Frangaj and Fan, 2018). Agonist activation is triggered by the closing of the VFT module, which occurs in the ligand-binding subunit $GABA_{B1}$, and both receptors (B1 and B2) dimerize to form the heterodimer interface (Geng, 2013). The formation of such a complex results in the initiation of G protein coupled signaling.

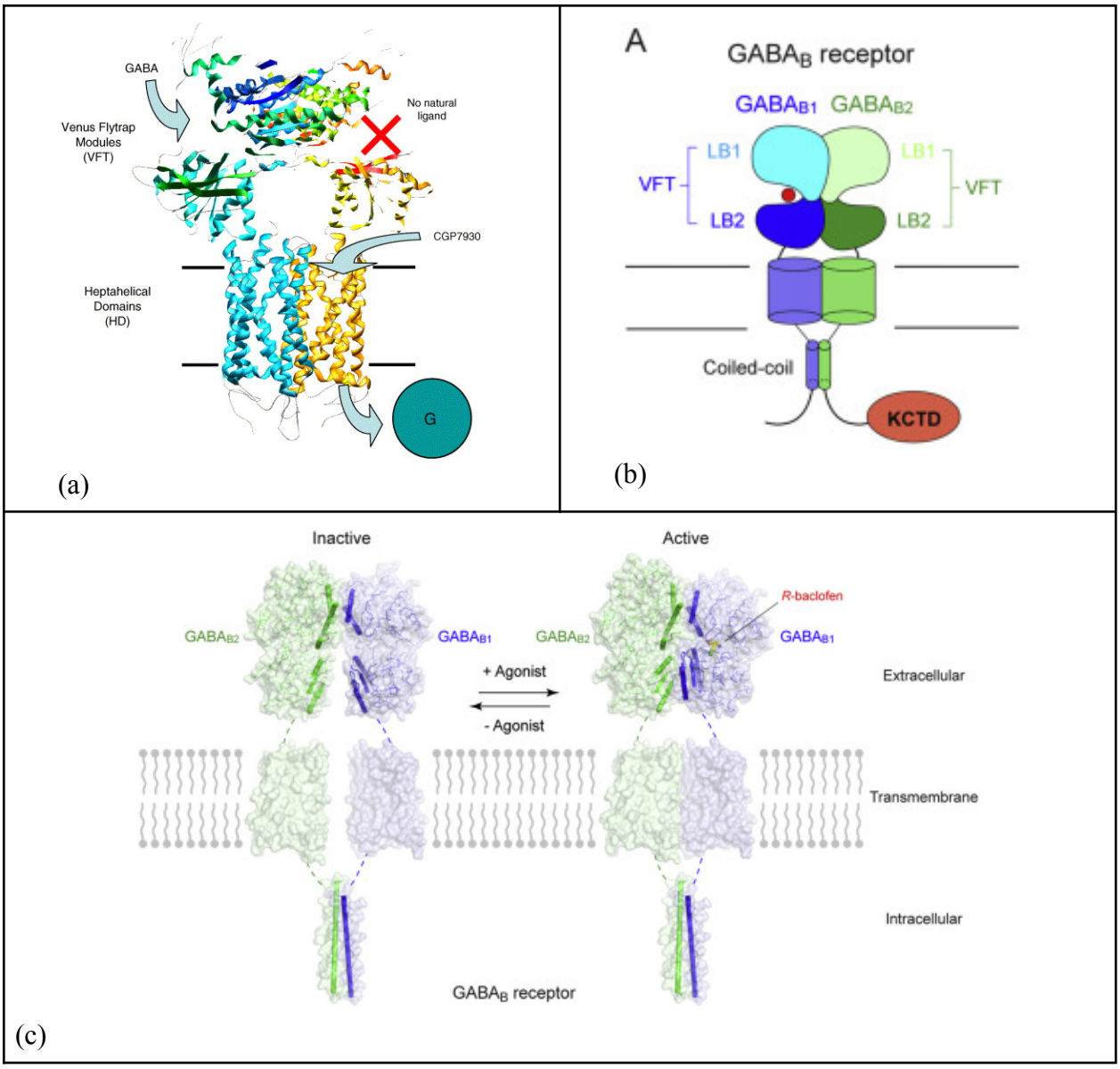

Figure 5: (a) The proposed structural organization of the heteromeric GABAB receptor. The GABAB1 subunit is in blue, in front, and the GABAB2 subunit in yellow, in the back. As shown, each subunit is composed of a Venus Flytrap (VFT) module directly connected to the heptahelical domain (HD) (b) Schematic representation of GABAB receptors (c.) Conformational equilibrium between the inactive and active states of GABAB receptor.

GABA synthesis

Distribution of $GABA_{B1}$ mRNA has been experimentally analyzed by *in situ* hybridisation in the model organism, the rat. $GABA_{B1}$ mRNA is detectable in almost all neuronal cell

populations, with highest levels of expression in hippocampus, thalamic nuclei, and cerebellum; high levels of GABAB2 mRNA are seen in the piriform cortex, hippocampus, and medial habenula ((**Figure 6;** Bettler 2004).

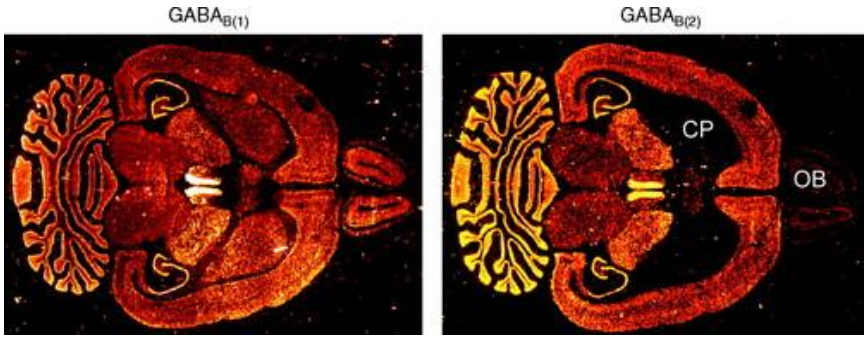

Figure 6. Comparison of the distribution of GB1 and GB2 subunit mRNA in the rat brain. both are expressed at similar levels throughout the brain, with few exceptions: GB2 transcripts are less abundant in the olfactory bulb (Bettler 2004)

Physiology

It is important to consider how GABA receptors are involved in regulating physiological outcomes because benzodiazepines and barbiturates, the drugs used in PAD, act upon these receptors. Thus, identifying how these receptors interact with important life sustaining systems in the body is critical to consider because it provides vital insight into how these systems can be disrupted in overdose and lead to death. For example, if we find that GABA receptors play an integral role in maintaining cardiac rhythm, we can hypothesize that PAD drugs would impair this ability. This is particularly useful in our case due to the ethical code preventing close monitoring of all vital functions during death via PAD, and the impossibility of investigating how the impairment of all of these mechanisms would lead to human death. As a result, there is

little information available. Thus, we must use the evidence gained as to how these drugs normally impair the system to generate theories as to how they may act in a fatal overdose circumstance.

Neurodevelopment

Studies on the development of embryonic cortexes suggests GABA plays a role in the proliferation of neural progenitor cells; GABA activation of $GABA_A$ receptors inhibits DNA synthesis of progenitor cells, reducing the total number of cells through depolarisation-inducing Ca2+ influx (LoTurco, 1995). Early excitation in the developing brain also influences the morphological development of neurons, resulting in reductions in neurite length, number of dendrites, and length of dendrites (Wu, 2015). The final significant role of GABA in neural development is that of modulating plasticity, a feature which has consequences on the resultant structure and connectivity of the adult brain; A significantly investigated area of this is the visual cortex. In the rat model, GABAergic inhibition results in changes to the structural development of cortical columns - the infusion of GABA agonists expanded the distance between cortical columns of the visual cortex where antagonists decreased distance (Vargas, 2018). The hypothesized implications of this are alterations in learning, memory, sensory processing, and motor processing.

Muscular

It has been discovered that α4 and β2/3 subunits of $GABA_A$ receptors are expressed in cholinergic neurons of sympathetic ganglia (Park 2016). Immunoreactivity to GABA has also been observed in human neuromuscular junctions and in the axons of motor neurones which result in inhibition of acetylcholine release from motor nerve endings (Nurulin 2018). GABA immunoreactivity was detected at all synapses (**Figure 7**). GAD enzymes involved in GABA synthesis were also observed. GAT-2, a GABA transporter, was visualized at all synapses (**Figure 7**). This highlights the role of GABA receptors in neuromuscular transmission. This is significant because of the presence of such synapses in systems critical in maintaining life such as the cardiac and pulmonary system.

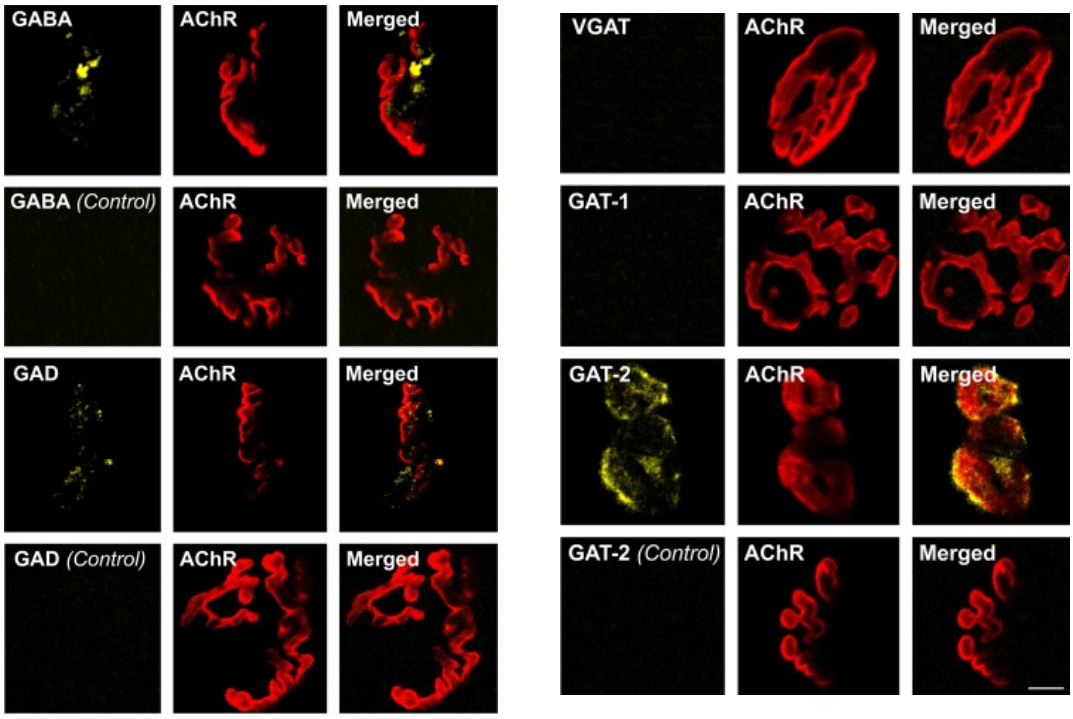

Figure 7. Micrographs from a rat diaphragm processed for immunochemical analysis with key antibodies against key molecules of the GABA ergic signaling cascade (GABA, γ-aminobutyric acid, GAD, L-glutamic acid decarboxylase, VGAT, GABA transporter 1&2). Red is illustrative of nicotinic acetylcholine receptors marking the postsynaptic membrane of skeletal muscle fibers and synaptic region. Antibodies against the GABA, GAD, and GAT-2 regions were highlighted in yellow (Nurulin 2018).

Cardiovascular regulation:

The results of model animal studies on cats injected with agents that either counteract the CNS effects of GABA or activate the CNS GABA receptors suggests evidence for the existence of tonically active GABAergic systems preset in the brain which exert inhibitory control over sympathetic activity (influencing arterial pressure and heart rate) (Gillis 1980). The same study also hypothesized the presence of a tonically active GABAergic system in the CNS that exerts

inhibitory control over spontaneously active parasympathetic neurons in cell bodies of motor nerves which innervate ipsilateral muscles. Neurones, via a GABergic synapse , inhibit sympathoexcitatory neurons in the ventrolateral medulla, a part of the medulla oblongata of the brainstem which is necessary for the role of regulating arterial blood pressure and breathing, demonstrating the role of neuronal action in baroreflex (the homeostatic mechanism maintaining stable blood pressure) (Dampney 2002). The site of action of this mediating effect is a discrete region on the surface of the brainstem which overlies a population of neurons thought to be the origin of basal sympathetic tone (Dimicco, 1987).

Pulmonary:

$GABA_A$ channels in the brainstem modulate cholinergic outflow to the lungs. In neural tissue activation of these receptors results in membrane hyperpolarization through a net movement inwards of Cl- ions (Mizuta 2007). This hyperpolarisation process, also known as $GABA_A$ mediated chloride entry, in smooth muscle membranes results in a decrease in intracellular calcium, and therefore a decrease in airway muscle tone (Mizuta 2007). This is because in reducing Ca2+ concentrations within the cell: the Ca2+ activation of myosin light chain kinase is reduced; resultant phosphorylation of myosin chains are reduced; and therefore so too are the interactions of actin and myosin, overall resulting in less muscle contraction

$GABA_A$ and $GABA_B$ receptors have been identified to inhibit cholinergic activity on the presynaptic side of the lung postganglionic parasympathetic nerves (Moore 2004). $GABA_B$-specific-agents decrease airway constriction by modulating acetylcholine release from

parasympathetic nerves and activation of metabotropic $GABA_B$ receptors inhibit acetylcholine release (Mizuta 2007, Malomouzh 2015).

Benzodiazepines

Benzodiazepines are psychoactive drugs typically prescribed due to their anti-anxiety, sedative, anti-convulsant, and muscular relaxant properties induced through their interactions with γ subunits of GABAa receptors - the CNS's major inhibitory neurotransmitter receptors (Rayes 2012). Benzodiazepines do not substitute for GABA, but instead enhance the inhibitory effects of GABA (Riss 2008). In general, benzodiazepines exhibit a rapid onset of action and enter the cerebral tissues rapidly, which is consistent with their short distribution half-lives (Rey 1999). Benzodiazepines cross the blood-brain barrier by simple diffusion to elicit their pharmacological effect and the onset of action is achieved by rapid distribution to the vessel rich brain (Rey 1999).

The basic chemical structure of benzodiazepines consists of a benzene ring fused with a seven-membered diazepine ring. A diazepine ring refers to a heterocycle with two nitrogen atoms, five carbon atoms, and the maximum number of cumulative double bonds possible. Benzodiazepine drugs then consist of a substituted 1,4-benzodiazepines (**Figure 8**) with the functional side-groups added to the structure modulating their resultant pharmacology through altering the binding of the drug to GABA receptors. Thus, due to this unique pharmaceutical profile, the potency, onset, and duration of action of benzodiazepines depend on their lipid

solubility, protein binding, and affinity for benzodiazepine receptors in the brain (Riss 2008). For example, the compounds midazolam, clonazepam, and lorazepam display greater affinities and lipophilicity than diazepam (Rey 1999). An increased rate of equilibration and faster onset of action is evident in midazolam and lorazepam compared to clonazepam and diazepam (Rey 1999). Similarly, there is a close relationship between the concentration of the benzodiazepines and their clinical effect: At low concentrations, anxiolytic and anticonvulsant effects dominate. As concentration increases, sedation and hypnosis become the principal clinical effects (Nillson 1991).

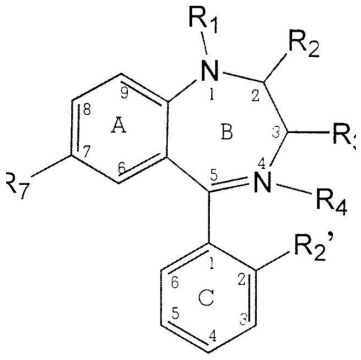

Figure 8: General chemical structure of 1,4- benzodiazepines.

Central Type vs Peripheral

Central-type benzodiazepine receptors are located primarily in neurons in the central nervous system and are macromolecular complexes that include a binding site for the inhibitory neurotransmitter GABA (Verma 1989). As such, they provide the mechanism of action for benzodiazepines via facilitation of the synaptic effects of GABA. Whereas peripheral-type

benzodiazepine receptors (PBRs) were discovered as benzodiazepine binding sites outside the CNS (Veeman 2006).

Peripheral benzodiazepine receptors are heterotrimers composed of three protein components: isoquinoline binding protein, a voltage dependent ion channel, and an adenine nucleotide transporter (Veeman 2006). Functions associated with PBR's are: regulation of steroid production, involvement in growth and differentiation, apoptosis, regulation of mitochondrial membrane potential, regulation of mitochondrial respiratory chain,immune response, modulation of calcium ion channels, ischemia (inadequate blood supply to an organ, in this case specifically the heart). Using rats, in vitro studies have found the binding density of PBRs to be greatest in the adrenal>kidney~heart>> liver and brain and further audiographic localizations found presence in the epithelium of the luing, lining of pulmonary arteries, thymus and bone marrow, as well as those locations listed previously (Veeman 2006).

Clinically potent benzodiazepines, like clonazepam, are believed to exert their action through central GABA receptor linked benzodiazepines binding sites. In contrast, Ro 5-4864, a benzodiazepine without anxiolytic effect, is found to be about 10,000 times more potent in peripheral Benzodiazepine binding sites (veeman 2006).

Benzodiazepine Affinity and mechanism

The mechanism by which benzodiazepines enhance GABA receptor function has been termed allosteric, that is, the diazepine binding site is distinct from that of the agonist (GABA) binding site (Campo-Soria 2006). Similar to the mechanism of the GABA agonist,

benzodiazepine, upon binding to the recognition site, results in a conformational change in the receptors, increasing the affinity for channel gating by GABA at the agonist sites, enhancing the receptor opening frequency (Sigel, 2012). However, whilst the functional GABA binding site on GABA receptors is located in the inter-subunit contact between α and β subunits, the amino acid residues of the α and γ subunits form the benzodiazepine binding pocket (Baumann, 2003). Newer, single channel studies have proposed further adaptations to the mechanism whereby diazepines increase the rate at which mono-ligand receptors open, rather than the enhancing affinity of the GABA model as previously thought (Campo-Soria 2006). The two proposals for this method are: (a) benzodiazepine and GABA site coupling such that diazepines alter the affinity of GABA binding sites, or (b) diazepine acts subsequently to the binding of GABA. Regardless of action on receptor kinetics, benzodiazepines do increase the conductance of individual $GABA_A$ receptors (Campo-Soria, 2006).

The binding of benzodiazepines is highly temperature dependent, with both the agonist and antagonist binding appearing enthalpy driven (Tallmaan, 1985). Benzodiazepines bind to a high affinity binding site located on the α/γ subunit interface of the $GABA_A$ receptor, this positioning is homologous to the agonist site (Sigel, 2012). Experimentation to identify the causes of binding affinity were conducted on human embryonic kidney cells which were transiently infected with cDNAs coding for various $GABA_A$ receptor subunits. The results of these investigations led to the conclusion that there are several amino acid residues on α-subunit iso-forms which have consequences on the binding affinity of ligands at the benzodiazepine site via their participate in the formation of the "binding pocket", specifically the amino acid residues aH101, aY159,

aT206, aY209, gF77, gM130 which are evidenced to increase binding ten-fold (Sigel, 1997) .Two types of benzodiazepine receptors have been described, central and peripheral. Central associates with $GABA_A$ receptors whereas peripheral ones are located on the outer-mitochondrial membrane and show no relation to $GABA_A$ receptors (Masahito 2002). Central benzodiazepine receptors demonstrate different affinities to different benzodiazepines, and upon this two further categorisations have been made; type I and type II. Type I receptors have increased sensitivity for CL21887 and 1,4-benzodiazepine-2-oxo-quazepam and are the dominant $GABA_A$/benzodiazepine receptors in the brain; In contrast, type II receptors are enriched only in the hippocampus, striatum, and spinal cord (Masahito 2002). The α and γ subunits are significant in that they are associated with the pharmaceutical properties of benzodiazepines; the α subunit in the type I receptor is α_1, those in type two are α_2, α_3, or α_5 (anti-anxiety, sedative, anticonvulsants etc.) (Rayes 2012, Masahito 2002).

Physiology of benzodiazepine induced death

Now that we have investigated Benzodiazepines pharmacokinetics and benzodiazepine receptors, it is important to consider the far reaching effects of such interactions on the grander scene of the body. That is what are the physiological changes occurring through benzodiazepine consumption on the; neuronal circuits, respiration, metabolism, cardiovascular system. Due to ethical considerations regarding the monitoring of vital mechanism during death in human subjects, little literature exists which thoroughly outlines the modes in which physiological changes contribute to death (or the hastening of death where conclusive mechanisms are known

to cause death such as the contributions of neurological systems in pulmonary edema). It is still important however, to consider how these elements may be at play between administration and death as, despite their significance, individuals will undergo these processes.“Traditional” benzodiazepine overdose presents itself in drowsiness, slurred speech, hypotension, ataxia, coma, and respiratory depression and hypoxia (Ramrakha 2004).

Cardiovascular

Peripheral-type benzodiazepine receptors (PBRs) are abundant in the cardiovascular system: in the cardiovascular lumen, PBRs are present in platelets, erythrocytes, lymphocytes, mononuclear cells, in the walls of the cardiovascular system, in the endothelium, the striated cardiac muscle, the vascular smooth muscles, and the mast cells (Veenman 2006). Functionally, PBRs in these locations are involved in apoptosis, cell proliferation, steroidogenesis, producing membrane potentials, ion channel regulation and PBRs specifically in blood vessel walls are involved in responses to ischemia - the PBR antagonist, SSR180575, was found to reduce damage correlated with ischemia (Veeman 2006). For example, an important modulator of cardiac action potential is the slowly activating delayed K+ current. In guinea pigs, benzodiazepine receptor agonists were found to activate this current and shorten action potentials in cardiac myocytes (Salata, 1998). Benzodiazepine agonists in a follow up study were found to inhibit the cardiostuimulany activity of a nitrendipine analogue which under normal circumstances, increases the conductivity voltage sensitive calcium channels (Mestre 1985).

In an experiment that tested whether GABA-enhancing effects of a benzodiazepine could be observed in the heart rate change (R-R interval) immediately following the onset of a brief (10s) isometric contraction of the biceps muscle, experimenters found significant results. To conduct the experiment, a comparison was made between the change in R-R interval occurring during the same phase of respiration for placebo and 10 mg oral diazepam treatment (Farmer 2003). Contractions initiated following diazepam treatment resulted in a significantly greater reduction in R-R interval implying that GABAergic suppression of cardiac vagal outflow may be responsible for contraction-induced tachycardia (heart rates over 100bpm; Farmer 2003).

Thus, I hypothesize the significance of this by proposing the following process. It is known that the shortening of cardiac action potentials result in increased inducibility as it promotes reentry and therefore further stimuli. Tetanic contractions are the result of sustained muscle contraction due to high frequency stimuli. Prolonged stimulation (comparable to tetanic contraction) in the heart leads to fast heart rhythm which results in cardiac arrest and death.

Pulmonary

In an experiment investigating the effects of benzodiazepines on breathing pattern and thoracoabdominal motion, eight healthy male volunteers in a randomized double-blind trial at 2 minute intervals injected with two doses of midazolam (0.05 mg/kg) or diazepam (0.15 mg/kg; Berggren 1987). The initial injection of both benzodiazepines caused significant decreases in tidal volume, inspiratory and expiratory time, while minute ventilation, mean inspiratory flow and respiratory timing were not significantly affected (Berggren 1987).

With respect to inducing death, I hypothesize that this process is significant as a reduction of tidal volume may result in the collapse of part, or the entirety of the lungs. This effect will be compounded by the decrease in inspiratory and expiratory time resulting in difficulty in saturating alveoli, and therefore a reduction in oxygen concentrations. It is likely that this would cause the cardiac system to work harder in order to provide oxygen to respiring vital organs. The heart working harder would increase necessary rates of respiration and therefore the necessity for oxygen which is already limited.

Neurology

An investigation into the benzodiazepine receptor using the agonist FG 7142, has shown that benzodiazepine receptor agonists modulate attentional and cognitive performance by their dampening effects on cortical acetylcholine release (Hart 1999). PBRs are key elements of cholesterol mitochondrial import. The receptor binds with high affinity to cholesterol, supplying the substrate to the first steroidogenic enzyme, initiating and maintaining neurosteroid synthesis (Papadopoulos 2006). Upon ligand activation, PBR cholesterol transport is accelerated thus increasing the formation of neuroactive steroids integral in normal brain function. These steroids are involved in many neurological disorders such as anxiety and mood disorders, therefore demonstrating that PBRs play a critical role in regulating brain dysfunction and further offer an opportunity for new therapies (Papadopoulos 2006).The Ca2+ induced permeability transition pore (PTP) is involved in mitochondrial events which lead to programmed cell death; an immunoblot revealed adenine nucleotide transporter, a key PTP component within the PBR

immunoprecipitate (Azarashvili, 2007). The resultant delay in calcium induced dissipation of membrane potential is indicative of suppression of PTP opening.

I hypothesize that these mechanisms are important in PAD in creating a biochemical-neurological environment under which individuals can relax in their final moments. The anti-anxiety qualities of PBR activation will be useful in easing the transition of life into death for patients, making the experience more comfortable both for patients and loved ones. This is because loved ones may be distressed at the prospect of the death of the patient, observing a peaceful, unconcerned passing will minimize stress.

Metabolism and respiration

Mitochondria are well known sites of respiration due to their location as sites of electron transport and therefore generators of ATP. PBRs are found to be expressed at high levels within the mitochondria, therefore it is critical to investigate their role in respiration. PK11195, an isoquinoline carboxamide, and Ro5-4864, a peripheral benzodiazepine receptor ligand, have been found to alter respiration with potencies correlated to their affinities for the PBR (Casellas 2002). As a result, PBR ligands were thought to increase state IV (oxygen consumption by isolated mitochondria in the presence of no oxygen) and state III (ADP stimulated respiration of isolated coupled mitochondria) respiration rates so that activation of the PBR significantly decreased the respiratory control ratio. Experimentation showed drugs that bound to the peripheral-type or mitochondrial benzodiazepine receptors in rat kidney mitochondria produced several effects on mitochondrial respiration. Indeed, the drugs increased state IV and decreased

state III respiration rates, resulting in a significant decrease in the respiratory control ratio suggesting that ligand binding to mitochondrial benzodiazepine receptors results in inhibition of mitochondrial respiratory control (Hirsch 1989).

Normally, inductors of mitochondrial permeability transition pore (MPTP) activity require accumulation of excessive calcium ion concentrations in the mitochondrial matrix, but PBR ligands can activate MPTP without further influx (Casellas 2002). MPTP plays a vital role in the biochemical functions necessary for maintaining cell vitality with the MPTP being considered the central executioner of cell fate following damage such as oxidative stress and exposure to cytokines (Casellas 2002).

As ligand binding to the PBR decreases respiratory control ratio and therefore inhibits mitochondrial respiratory control, we can hypothesize that this contributes to death via a significant decrease in ATP production. As ATP is the predominant compound necessary in supplying the energy to sustain contraction of the cardiovascular system, and muscular components of the pulmonary system, a reduction in ATP would impair function. As described above, impairment of these vital systems will result in death. It is possible that these effects will be compounded by MPTP due to induced mass cell death resulting from oxidative stress and cytokines released as systems of the body fail.

Muscular

The effects of the benzodiazepine midazolam were further studied on frog skeletal muscle fibers held under voltage-clamp conditions. Midazolam induced a concentration-dependent

(10−5 mol/1 to 10−3 mol/1) block of action potentials, specifically due to blocking of the underlying sodium current (Duval 1993). The resultant shift to negative potentials due to the inactivation of sodium channels allowed for the dissociation constant of midazolam to be calculated at 6.0 μmol/1, indicating that midazolam blocks inactivated rather than resting Na+ channels (Duval 1993). This mechanism of action might contribute to the well-known myorelaxant effect of the benzodiazepines. **Figure 9** illustrates the neural regulation of muscle tone, and indicates which areas benzodiazepines act upon to enact muscular relaxant effects.

Effects of diazepam on neuromuscular transmission blocked by neostigmine were also studied in isolated mouse phrenic nerve-diaphragm preparations. At concentrations of 175 mM or higher, diazepam caused blockage of axonal conduction (Chiou 1993). The conclusions of the investigation suggested that benzodiazepines reverse the muscle paralysis induced by anticholinesterase agents by inhibiting the regenerative release of acetylcholine (Chiou 1993). Diazepam is thought to improve the peripheral neuromuscular transmission that anticholinesterase agents inhibit -this process results in muscle contraction.

The myorelaxant effects of benzodiazepines are significant in the process of PAD as it ensures that individuals will be relieved of discomfort in their final moments. Muscle relaxants also prevent unwanted symptoms prior to death such as vomiting, this is because vomiting occurs due to the contraction of the diaphragm, the intercostals, and abdominal muscles. The tertiary effect of muscle relaxants relates to the heart, as the heart is a muscle, muscular relaxants will also slow down heart rate leading to heart failure.

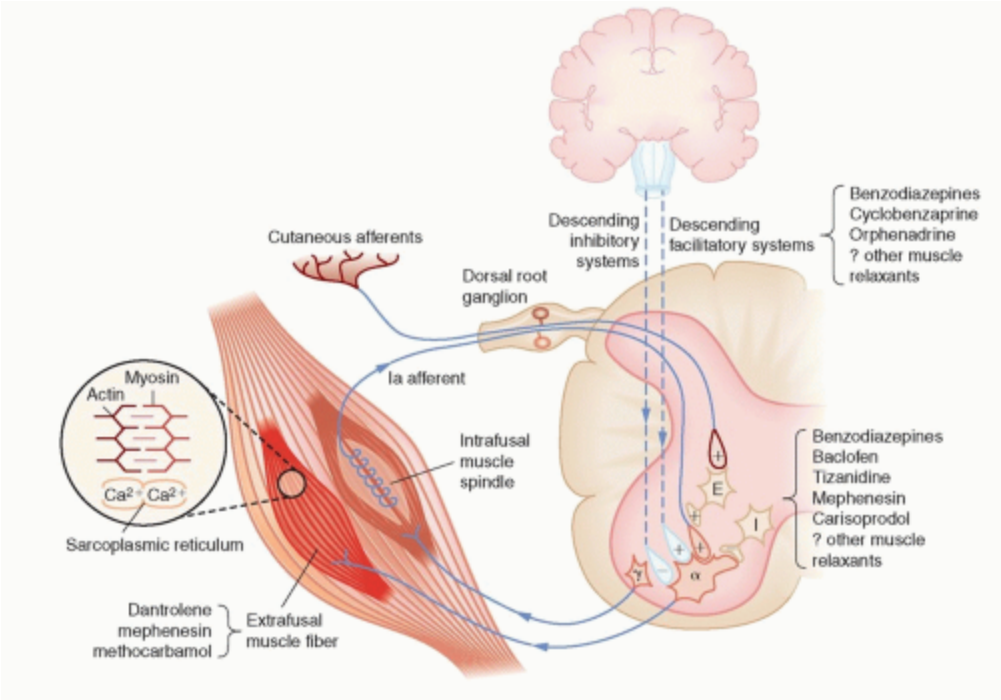

Figure 9: Neural regulation of muscle tone: possible sites of action of skeletal muscle relaxants. See text for details. +, excitatory effect; -, inhibitory effect; α, α-motoneuron; Ca2+, calcium ion; E, excitatory interneuron; γ, γ-motoneuron; I, inhibitory interneuron. (Chaghtai 2020)

Terminal sedation

Benzodiazepines are administered in PAD due to their role in terminal sedation and ability to induce comas (Quill, 1997). In hepatic comas, the number of benzodiazepine and GABA binding sites in the brain increases (Lal 1987). There is, however, uncertainty about the required dose and duration to ensure a comatose state. The correct dosage is determined to be the point in which the patient becomes unconscious, but given a high degree of uncertainty and individual variation, drug administration may take days or even weeks to result in death, a major reason for using cocktails of multiple drugs (Quill, 1997). In an experiment in which eighteen non-intubated

patients were poisoned with hypnotic benzodiazepine sedatives in order to measure neurological and respiratory changes, experimenters found drug-induced coma to be characterized by snoring, flow limitation, and obstructive apnea (Gueye, 2002). Thus, a direct consequence of the comatose state is an increase in upper airway resistance and more difficulty breathing. Ultimate brain death is characterized by complete and irreversible coma, absent brainstem reflexes and apnea (Machado 2010). The two mechanisms of injury in order to fulfil the categorisation of being 'brain dead' are an elevation of intracranial pressure beyond arterial pressure leading to permanent cytotoxic injury of neuronal tissue and injury of tissues at a cellular level (Machado 2010).

Barbiturates

Barbiturates are administered for physician-assisted euthanasia, however their use in non-PAD, clinical practice has largely been replaced by combinations of barbiturates with benzodiazepines such as alprazolam, diazepam, and lorazepam due to the lower risk of overdose and antidote to reverse toxicity (Suddock 2021). Despite the prevalence of barbiturates declining, an understanding of barbiturates pharmacokinetics and mechanisms is still necessary and important to understand due to their history of being administered independently and because of their role in the PAD barbiturate-benzodiazepine combination leading to death.

The development of the class of barbiturates began with the synthesis of barbituric acid by the German chemist and Nobel Prize winner Adolf von Baeyer (Loscher 2012). Barbituric acid itself is ineffective to the CNS due to its insufficient lipophilicity and inability to penetrate the blood-brain barrier. Thus subsequent chemists would later alter its composition, eventually leading to the production of Pentobarbital (Loscher 2012). Barbiturates have in common the pyrimidine-2,4,6-trione structure (**Figure 10**). Barbiturate sedatives contain heterocyclic, six-membered rings, featuring a highly selective affinity for forming hydrogen bonded complexes with molecules containing adenine which is integral in their binding to membrane lipids (Ho, 1981).Barbiturates are CNS depressants that are used as sedatives, hypnotics, anesthetics and anticonvulsants, and their primary site of action is the GABAa receptor (Ito, 1996). The concentration of barbiturates has consequences on GABA receptor action such that at low concentrations, barbiturates positively modulate GABA receptor response via an allosteric mechanism which increases hyperpolarization of postsynaptic membranes and at high concentrations barbiturates are GABA-mimetic, activating GABA receptors even in the absence of GABA (Costa, 1979). Thus, at lower concentrations barbiturates act via modulating the GABA receptor and changing its function and at higher concentrations, act as agonists, activating the receptor itself to produce a biological response.

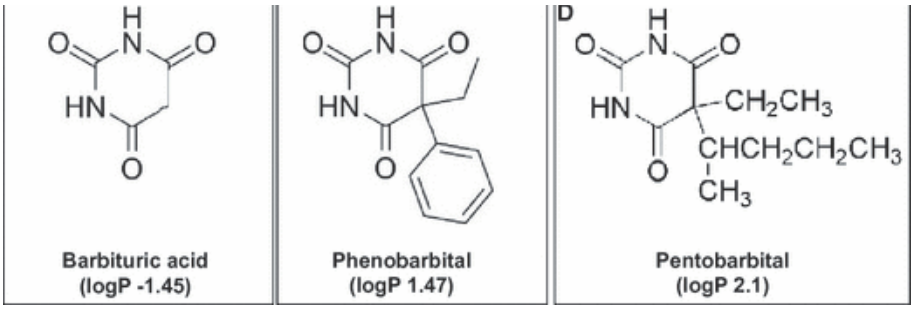

Figure 10: General chemical structure of Barbituric acid, phenobarbital, and pentobarbital.

Barbiturates Affinity and Mechanisms

Barbiturates interact with α and β subunits of the GABAa receptor; this interaction results in the postsynaptic enhancement of GABA. Barbiturates do this by increasing chloride ion influx which results in dose dependent, GABA-induced postsynaptic inhibition (Skibiski 2021). Dosage influences the action of barbiturates on $GABA_A$ receptors as doses associated with anesthetic effects are high and these doses are associated with the direct activation of GABA receptors. Lower doses of barbiturates act by prolonging and potentiating the action of GABA on GABAergic receptors (Loscher 2012). Whilst both barbiturates and benzodiazepines interact with GABAa receptors, barbiturates are unique in the fact that they potentiate the receptors whilst increasing chloride ion influx even under low concentrations of GABA neurotransmitter (Skibiski 2021).

The interaction of barbiturates with membrane lipids offers another plausible mechanism as to how barbiturates cause changes in neuronal function. Barbiturates are known to perturb the ordering of membrane lipids and acyl head movement. Such findings suggest the possibility of altering the biophysical characteristics of the synaptic membrane which may influence $GABA_A$

receptor binding (Ito 1996). This is because altering these properties have been shown to modify the activity of membrane bound enzymes like protein kinase C, which may have consequences on $GABA_A$ receptor functioning. Therefore as lipophilicity of barbiturates increases, the inhibitory potency increases (Ito 1996).

Barbiturates also affect the transport of calcium in excitable tissues and block the accumulation of depolarization induced increases in calcium in the ganglia and brain synapses of squid (Ho, 1981). The effects of this process mediate the anticonvulsant effects of barbiturates as supported by observations that nonbarbiturate anticonvulsants inhibit calcium uptake and convulsant barbiturates show anticonvulsant activity (Ho, 1981). Given that barbiturates inhibit calcium accumulation in nerve endings, it is also observed that barbiturates inhibit the release of acetylcholine. In experiments aimed at understanding why twitching occurs with the onset of barbiturate anesthesia, experimenters found that barbiturates enhance the resting release of neurotransmitters by reducing the surface charge on synaptic vesicles and inhibiting intra synaptosomal sequestration of calcium (Ho, 1981).

Physiology

Now that we have investigated barbiturate pharmacokinetics, it is important to consider the far reaching effects of such interactions on the grander scene of the body. That is what are the physiological changes occurring through barbiturate consumption on the cardiovascular, pulmonary, and metabolic systems. It is important to note that barbiturates are rarely recorded as administered independently of other drug classes, therefore the information available outlining

the singular effects of barbiturates is limited. Beyond the analysis into pulmonary death, little literature exists on how other physiological methods contribute to death due to ethical constraints. It is necessary and important, to possess a thorough understanding of these processes regardless as they may still be implicated in physiological changes which contribute to death - i will hypothesize their roles in this section.

Similarly, as the use of barbiturates has declined due to associated risks of administration, many of the studies outlined in this section are from the 1940s-80's and therefore comprehension may have changed. Generally, toxicity of barbiturates are marked by: difficulty thinking, decreased consciousness, bradycardia, rapid and weak pulse, poor coordination, vertigo, nausea, muscle weakness; fatal cases are marked by coma, hypotension, respiratory depression (Suddock 2021).

The progressive deterioration of the ballistocardiogram of the anesthetized dog is a reflection of anesthesia-induced circulatory depression resulting in pooling of blood in extrathoracic areas, and reduction in venous return, cardiac output, and arterial pressure. Possible PAD related significance is that of death by bodies organs not receiving enough oxygenated blood (resulting in shock) brought about by a decrease in blood pressure.

Barbiturates are particularly potent due to their ability to increase chloride influx in the absence of GABA, a quality which accounts for their ability to significantly depress the central nervous system, contributing to their toxicity (Suddock 2021). Anesthetics depress the central chemical mechanism of breathing, specifically carbon dioxide control, much more rapidly than that of peripheral reflex chemical mechanisms such as carotid and aortic chemoreflexes, and

vago pulmonary proprioceptive reflexes (Moyer 1942). In a study investigating the mechanisms leading to respiratory arrest and death, researchers conducted an analysis of evipal and pentothal anesthesia. There is a reciprocal relationship between dosage and pulmonary respiration, where increasing dosage leads to respiratory failure. Barbiturates rapidly diminish, and ultimately eliminate, the ability of the respiratory center to sense low levels of carbon dioxide known as central depression (Beecher, 1941). Under normal conditions, any depression to the central drive mechanism will be counteracted by the opposing drive of the reflex system (**Figure 11**). The reflect system is a mechanism which detects low oxygen levels and stimulates reflexive breathing. The depression of the central drive mechanism surpasses that of the reflex drive system. Respiratory depression occurs as reflexes driving manual respiration are inadequate to maintain oxygenation (Beecher 1941). Furthermore, as the body is no longer able to detect concentrations of carbon dioxide in the blood and dispose of it correctly, CO2 builds up in the blood until it too, acts as a depressant. Respiration fails, and blood pressure falls - withstanding any artificial respiration, the individual will die (Beecher 1941). It is the culmination of the depressive effects of low oxygen, high carbon dioxide, and the depressive action of the barbiturate itself which leads to fatality. Studies into the high-dose, oral administration of short acting barbiturate pentobarbital showed the following physiological changes: firstly, a comatose state, followed by a decrease of cardiac output, until ultimately the process outlined above leads to respiratory arrest (Sumner 1975).

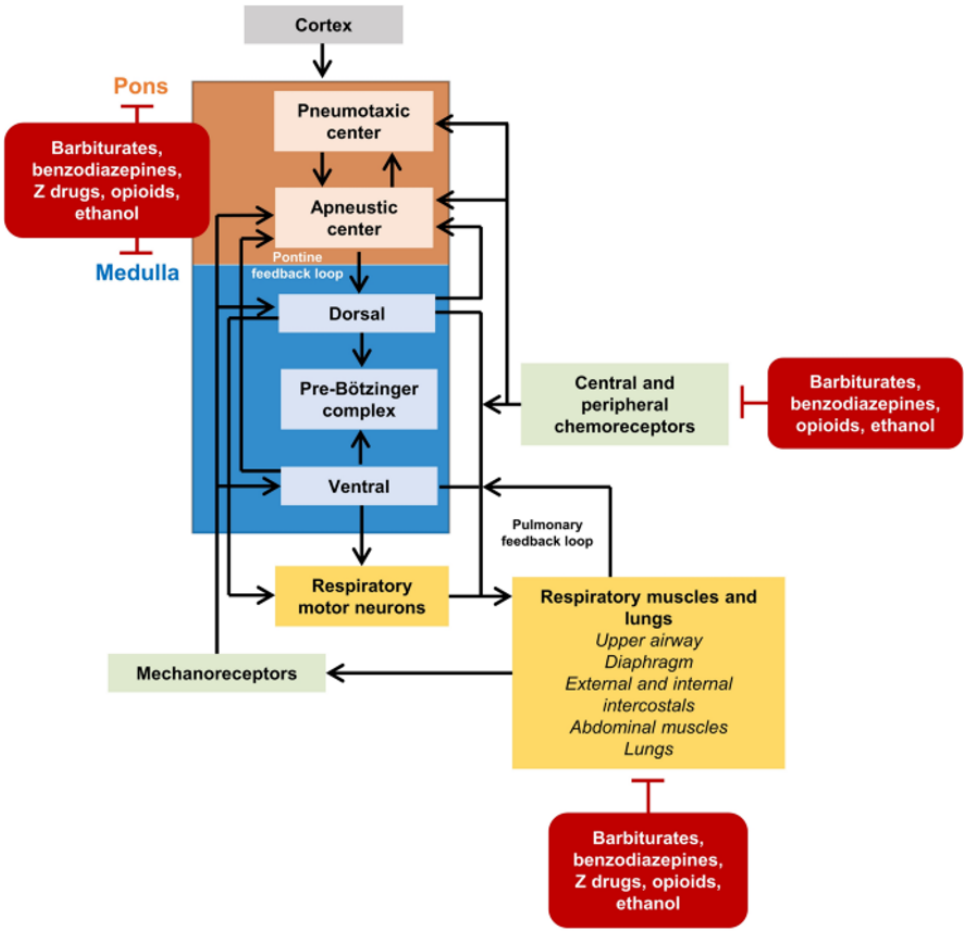

Figure 11. Effect of CNS depressants on respiration. Pharmaceutical and recreational agents such as barbiturates, benzodiazepines, Z drugs, opioids, and ethanol can suppress multiple steps in respiration to cause respiratory depression (Webster 2020)

Barbiturates inhibit mitochondrial respiration by potentiating NMDA receptor-mediated neurotoxicity at excitatory synapses (Anderson, 2002). Experiments using rat cortical cultures examined the effect of barbiturates secobarbital, amobarbital, and thiamylal, on neuronal mitochondria and NMDA receptor stimulation (Anderson, 2002).Researchers found that barbiturates caused depolarisation of neuronal mitochondria caused by ATP synthase inhibition,

suggesting that this mode of action involves inhibiting electron transport to cause ATP synthase reversal (Anderson 2002). Barbiturates also inhibit succinate oxidation in tightly coupled mitochondria. As explained previously when discussing benzodiazepine physiology, a decrease in ATP synthesis has a domino effect on the bodies ability to regular life-sustaining systems, specifically pulmonary and cardiac systems which leads to death.

Opioids

Opioids are frequent drugs of choice by pain specialists and addicts alike, due to their rapid onset of action and efficacy(Connor, 2002). Opiates act in both the central and peripheral nervous system (PNS). In the CNS, opioids primarily affect the spinal cord and in the PNS, these drugs influence the myenteric plexus and submucosal plexus in the wall of the gut and joints (Chahl 1996). One of the most investigated uses of opioids regards their role in pain relief. In pain reduction, opioids inhibit neurotransmitter release from dorsal root ganglion projections in the dorsal horn of the spinal cord (Jordan 1998). The agonist binding and subsequent receptor activation initiates a cascade resulting in analgesia, miosis, bradycardia, general sedation, hypothermia, insensitivity, and depression of flexor reflexes (Jordan, 1998).

To investigate the pharmacology of opiates, I will focus on a commonly used drug for PAD, morphine (**Figure 12)**. Morphine is the prototypical M receptor opioid derived from phenanthrene (Trescot, 2008). After administration, approximately 45% of the dose reaches the

CNS in 30 minutes. Compared to more modern opioids,this rate of penetration is categorized as poor and is due to the low lipid solubility, receptor affinity, glucuronic acid conjugation , and frequent ionization at physiological pH characteristic of morphine (Trescot, 2008).

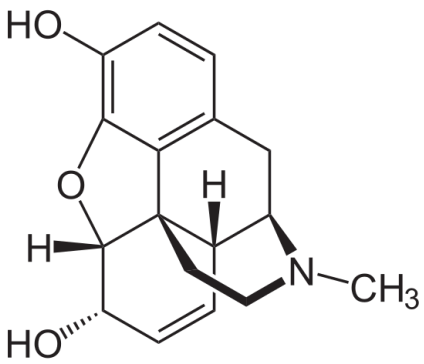

Figure 12. The chemical structure of morphine, the prototypical opioid (Ogura, 2013)

Opioid drugs produce their pharmacological effects by acting on receptors located on neuronal cell membranes (Chahl, 1996). The biological effects exerted by opioid receptors are initiated by the formation of receptor-ligand complexes (Jordan 1998).These receptors exhibit their cellular effects via the activation of, and coupling to, heterotrimeric G-proteins (Connor 2002). Three types of receptors have been identified based on their affinities for the (-) as opposed to the (+) stereoisomer of opioid alkaloids. These include the μ (also known as OP3) and κ receptors (OP2) and are defined based on their differing action upon binding their respective prototypic agonist. Additionally, the δ receptor (OP1) is defined by comparing the activity of endogenous opioid peptides and opioid drugs in a variety of systems (Connor 2002).

Figure 13 reflects opioid receptor localization based on receptor binding, in situ hybridization, and the localization of fluorescently tagged receptors in genetically modified mice (Valentino 2018). Taken together, alongside experimental data outlining cellular and circuit responses to receptor activation, these findings highlight how opioids produce their effects (Valentino, 2018). Each receptor mediates a particular analgesic effect. μ receptors have been correlated to the qualities of sedation, vomiting, respiratory depression, pruritus, euphoria, anorexia, urinary retention, and physical dependence, δ receptors to brain and spinal analgesia, and κ receptors to analgesia, sedation, dyspnea, psychomimetic effects, miosis, respiratory depression, euphoria, dysphoria, dyspnea (Trescot, 2008).

An experiment using single amino acid substitution allowed investigators to determine the critical amino acids necessary for agonistic and antagonistic binding. The results of this experiment found aromatic transmembrane residues at positions 129 and 308 and amino acids at position 95, 284, 296, and 297 of the δ receptor necessary in binding to ligands but more evidence was necessary in finding further critical residues for receptor activation (Jordan, 1998).

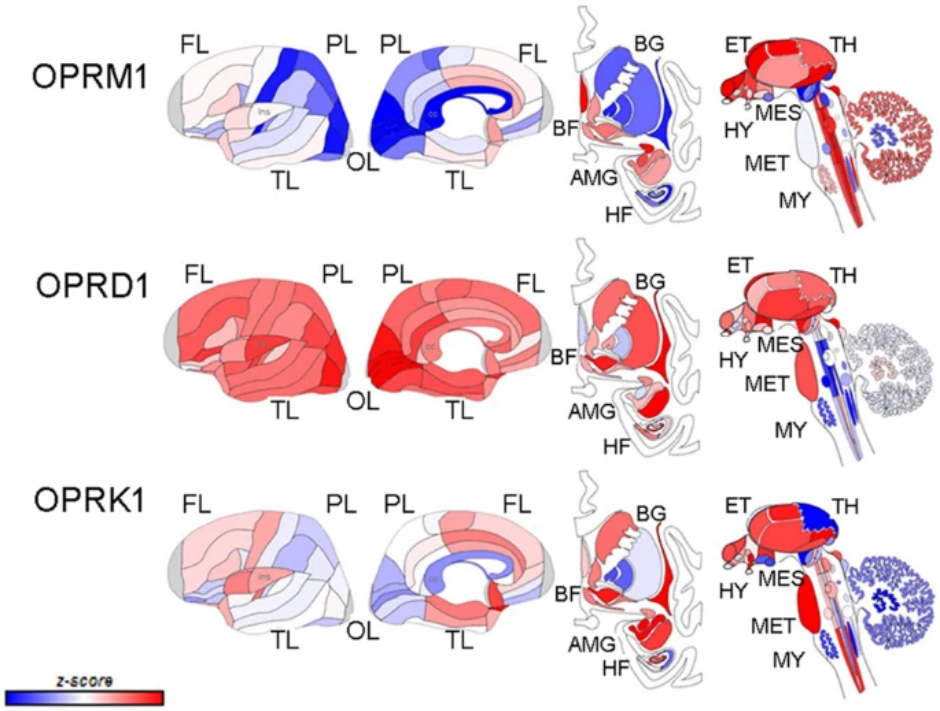

Figure 13. A schematic depicting the differential location of μ (OPRM1), δ (OPRD1) ,and κ (OPRK1) based on gene expression patterns in the human brain. The first two columns are reflective of the outer and inner surfaces of the left hemisphere. The third column reflects the subcortical structures from the frontal view. The fourth column shows the subcortical and brainstem structures.(Valentino 2018).

Mechanism

The major effect of opiates on the nervous system has been defined as their role in presynaptic action inhibiting neurotransmitter release.In order to comprehend the action of opioids and how their administration brings along physiological changes, we must first understand what G-protein-coupled-receptors are and how they are functionally relevant. G-proteins are characterized by their structure which considest of seven putative transmembrane

domains , intracellular domains between the 5th and 6th transmembrane segments, an extracellular N-terminal, and an intracellular C-terminal domain (Connor, 2002). Opioid receptors belong to a group of receptors that interact preferentially with the pertussis toxin-sensitive G-proteins of the G_i and G_o families. As a result, opioid signals are blocked by the pertussis toxin, a bacterial toxin, which ADP-ribosylation and inactivates the alpha subunits of G_i-G_o proteins (Connor, 2002).

At rest, guanosine diphosphate (GDP) associates with the a-subunit, upon opiate binding, GDP dissociates from the a-subunit and guanosine triphosphate (GTP) assumes its position producing a conformational change which causes the opioid to dissociate (Chahl 1996). As opioid receptors are coupled to potassium channels and calcium voltage gated channels due to their association with G-proteins, opioids are capable of inhibiting the release of neurotransmitters (Chahl 1996). This is because the release of neurotransmitters is dependent on the depolarisation of the nerve terminal and calcium ion entry through voltage sensitive calcium ion channels. Opiates directly inhibit N-type Calcium ion channels and stimulate them to open voltage gated potassium ion channels inducing hyperpolarisation thereby preventing excitation or propagation of the action potentials (Chahl, 1996; Connor, 2002). It is also hypothesized that opioids play a third role in reducing neurotransmitter release due to their relation to adenylate cyclase. Adenylate cyclase is involved in the hydrolysis of ATP into cyclic adenosine monophosphate (cAMP), a compound suspected to inhibit neurotransmitter release (Chahl, 1996). Opioid agonists activate opioid receptors located on the presynaptic terminals of the nociceptive C-fibers and A delta fibers and indirectly inhibit voltage-gated calcium channels,

decreasing cAMP levels and blocking the release of pain neurotransmitters like glutamate (Trescot, 2008). The inhibition of cAMP production has secondary effects also. By inhibiting the production of cAMP, opioids can alter protein phosphorylation, this is due to the role of cAMP as 'second messengers' whose role it is to activate protein kinases, which can lead to protein translation and gene transcription (Trescot 2008; **Figure 14**). The fourth and final way opioid receptors cause changes intracellularly, is via increasing intracellular calcium levels. Activation of δ-opioid receptors in neuroblastoma/glioma hybrid cells stimulates myo-inositol/phospholipase C, a factor mediating cell signal transduction, 1,4,5-triphosphate (IP3) signaling molecule formation, and subsequent Ca2+ mobilization (Connor, 2002, Jordan, 1998).

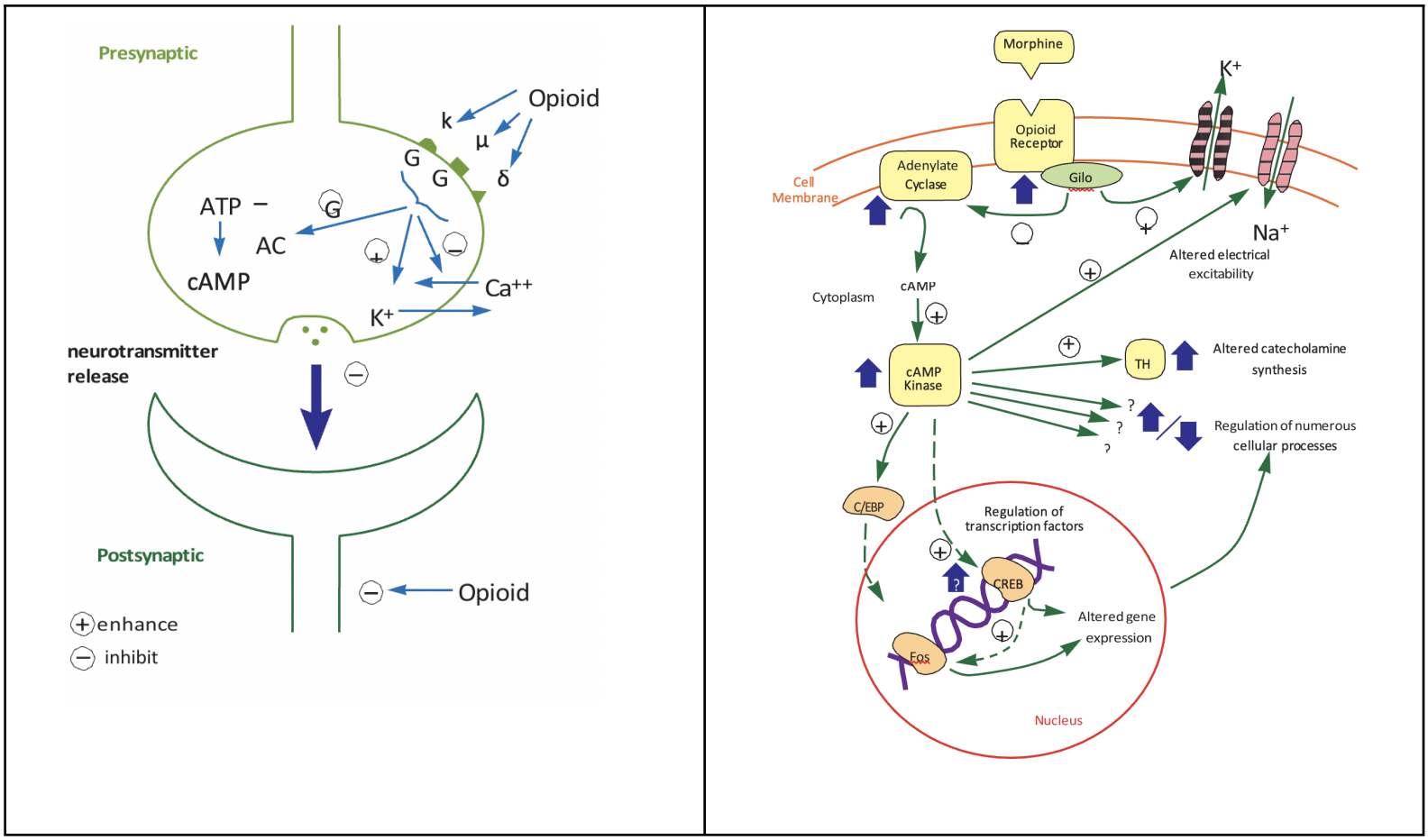

Figure 14. A summary of the action of the opioid morphine on the cell through ion channels and adenylate cyclase (Trescott 2008).

Physiology of Opioid induced death

Given that we have now established the pharmacokinetics and mechanisms of opioid action, it is important to consider the effects of opioid use on the cellular level. The following section will investigate what physiological changes occur through opioid administration on the; cardiovascular, pulmonary, neuronal circuits, muscular, and metabolic systems. Due to ethical

implications which prevent invasive monitoring of mechanism during the elapsing of death in human subjects, literature which thoroughly outlines the modes in which physiological changes contribute to death (or the hastening of death where conclusive mechanisms are known to cause death such as the contributions of neurological systems in pulmonary edema) does not exist. It is, however , still important to possess knowledge regarding opioid action in these systems so that we can hypothesize how these mechanisms may be extrapolated in the event of death.

Cardiovascular

Opioid receptors have several roles in cardiovascular pathophysiology, including hypertension, heart failure, and ischaemic arrhythmogenesis through direct and indirect mechanisms of action (Pugsley, 2002). Endogenous opioid peptides have been found distributed throughout cardiac nerves, intrinsic nerves of the gastrointestinal tract, and in cardiac muscle in the heart. Myocardial cells in particular are capable of synthesizing, storing, and releasing such peptides (Pugsley, 2002). By processing mRNA of the cardiac peptide, experiments on adult rats found the distribution of these peptides to exist primarily in the atrial tissues (Pugsley, 2002). In neurons, opioid peptides are found within vesicles in the atria. A study of the action of these peptides showed δ- and κ-opioid receptor agonists are capable of directly depressing cardiac function (Vargish, 1989). In the heart, κ and δ receptor agonists inhibit atrial contractility, while agonists inhibit ventricular contractility (Barron, 1999). The differential distribution of these receptors suggests they participate in particular functions within cardiac tissue homeostasis (Pugsley 2002; **Figure 15**). As opiates block the propagation of action potentials in nerves, they

are also capable of blocking action in cardiac muscle preparations through similar inhibition of voltage-dependent sodium and potassium ion currents (Pugsley 2002).

In dogs under anesthetic, the administration of κ opioid receptor agonists resulted in a dose-related decrease in blood pressure, heart rate, peak systole, and cardiac contractility (Hall, 1988). These findings suggest an effect on reflex mechanisms regulating functions like heart rate or direct actions on electrical and mechanical processes. The consequences of the known action of opioids, that is, the blockage of ion channels, results in visible changes to ECGs indicating the drug's interaction with cardiac ion channels such as blockades to Na+ and K+ ion channels (Pugsley 2002).

Consequences on the viability of life are obvious here, by inhibiting the propagation of action potentials and therefore depressing contractility, the function of the heart will be impaired. The ability of the heart to contract its chambers will prevent the circulation of oxygenated blood to tissues maintaining life. A lack of oxygen accessibility in these tissues will block respiration. As a result, these tissues will die and be unable to fulfill their role resulting in death (PAD)

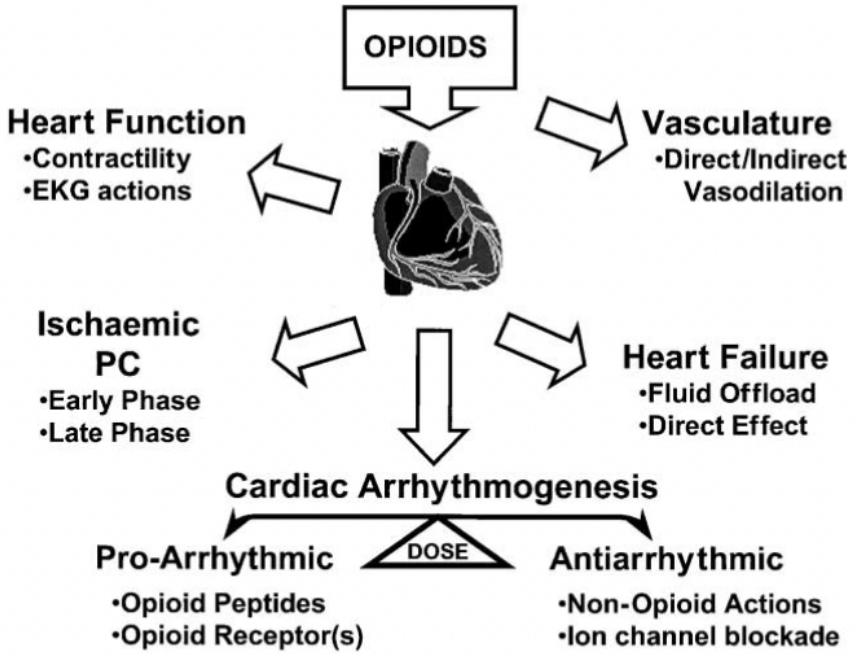

Figure 15. actions of opioids on the heart and cardiovascular system. (Pugsley, 2002)

Pulmonary

The lethality of opioids is largely due to the depression of the respiratory center, which occurs almost exclusively in opioid-naive patients (Willems, 1999). Respiration is controlled principally through medullary respiratory centers with peripheral input from chemoreceptors (**Figure 16(b)**). Opioids produce inhibition at the chemoreceptors via μ-opioid receptors, and in the medulla via μ and δ receptors (White, 1999). Respiratory drive is generated in the brainstem and is modulated by two inputs. First, the conscious input from the cortex and brainstem. Second, the peripheral input via chemoreceptors which sense changes in the chemical constituents of blood (Pattinson, 2008). μ, δ, and κ opioid receptors have been identified in these regions and react to both endogenous and exogenous agonists and affect respiratory drive

(Yamanaka, 2012). The most opioid sensitive aspect of respiration is rhythm generation, with opioids causing an irregular respiratory pattern after administration (Pattinson, 2008, **Figure 16 (a)**). The proposed mechanism for this action as modeled in rats is as follows: In the ventrolateral medulla, the activity of the pre-Bötzinger complex is inhibited by opioids. When this complex is depressed, rhythm generation is taken over by the retrotrapezoid parafacial respiratory group (Janczewski, 2006). This results in the redundancy of the rhythm generating centers in the brainstem causing respiratory slowing and arrest (Yamanaka, 2012). Further investigations into opioid action on central chemoreceptors has been somewhat limited, but studies which do exist suggest that localized application of opioids to areas of the brainstem have depressive effects on respiration (Pattinson, 2008, **Figure 16 (a)**). The consequences of the irregularity and slowing of respiration by opioids leads to hypercapnia, hypoxia affects tidal volume and rate (Yamanaka, 2012). Opioids also have been recorded to have a direct effect on airways leading to bronchoconstriction due to receptor presence in bronchial epithelium nerve fibers, and glands within the bronchial wall (Yamanaka, 2012).

Pulmonary conditions are the most common recorded complications of death , the most widely reported of which is non-cardiogenic pulmonary oedema (NCPE). The use of opioids including morphine, heroin and codeine, have been associated with development of pulmonary oedema (Yamanaka 2012). Consequences of opioid-induced NCPE include acute onset of hypoxic respiratory failure due to shunting occurring 12-24 hours after use (Yamanaka 2012). This circumstance occurs due to the imbalance between hydrostatic and osmotic pressure

resulting in net positive pressure out of the capillaries, an increase in capillary permeability, or both.

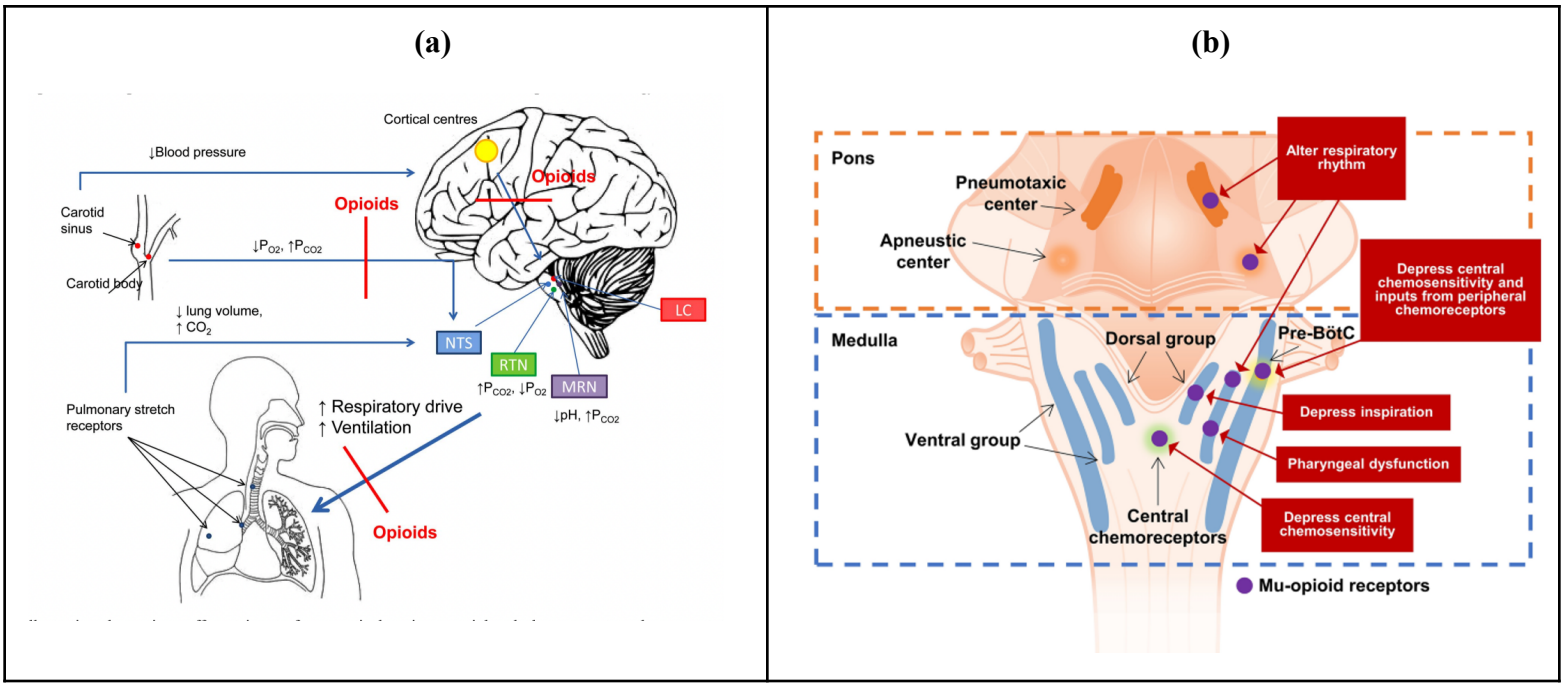

Figure 16. (a) Diagram illustrates the various afferent inputs from cortical regions, peripheral chemoreceptors, baroreceptors, pulmonary stretch receptors, as well as central chemoreceptors on respiratory drive and ventilation. The mechanisms of Opioid- induced respiratory depression are marked in red. (Koo 2011). **(b)** Opioid effects on central neural control. Opioids interact with mu-opioid receptors located throughout the respiratory centers in the brainstem, which under certain circumstances may suppress various components of respiratory drive. *Pre-BötC* prä-Bötzinger complex (Webster 2020).

Neuronal

As explained previously, the analgesic effects of opioid drugs are due to their binding and activation of opioid receptors. The opioid receptors respond to endogenous opioid peptides which are expressed throughout the peripheral and CNS and are key in modulating pain response. There are three particular areas of importance, the medial central amygdala (CeM), the periaqueductal gray (PAG), and the rostral ventromedial medulla (RVM). The CeM is responsible for the production of analgesia (Oliveria, 2001). The PAG is significant in that its role is to modulate behavior (pain, threat, and stress), cardiovascular control, and respirational control (Bagley, 2020) and the RVM provides the output from the pain modulatory circuit to the spinal cord (Heinricher, 2013). The most frequent receptor in the CeM is the μ opioid-receptor, its activation inhibits GABA release in this region (Bagley, 2020; **Figure 17**, blue). On GABA terminals, we also see the expression of κ opioid receptors (**Figure 17**, green). The most common receptor found in the PAG is also the μ opioid-receptor (**Figure 17**, blue) which is found to be expressed in neurons and presynaptic glutamate and GABA terminals. These receptors hyperpolarize neurons via coupling with G-proteins to influence the opening and closing of potassium ion channels and inhibit neurotransmitter release (Bagley, 2020). In this region, the presence of κ opioid-receptors (**Figure 17**, green) in presynaptic terminals and δ opioid-receptors (**Figure 17**, pink) on enkephalin terminals, has been confirmed. In the RVM, ON-cells are directly hyperpolarized by μ receptor agonists, OFF-cells are inhibited by κ receptor agonists, and both receptors inhibit presynaptic glutamate and GABA release (Bagley, 2020).

Neuronal changes induced by opioids are significant in the process of PAD due to their analgesic effects. This is specifically significant in increasing comfortability of patients as they transition from life into death, thus preventing trauma associated with experiencing pain or stress. Explanation of the significance of alterations to regulatory regions in the brain responsible for cardiac and pulmonary homeostasis have been explained previously.

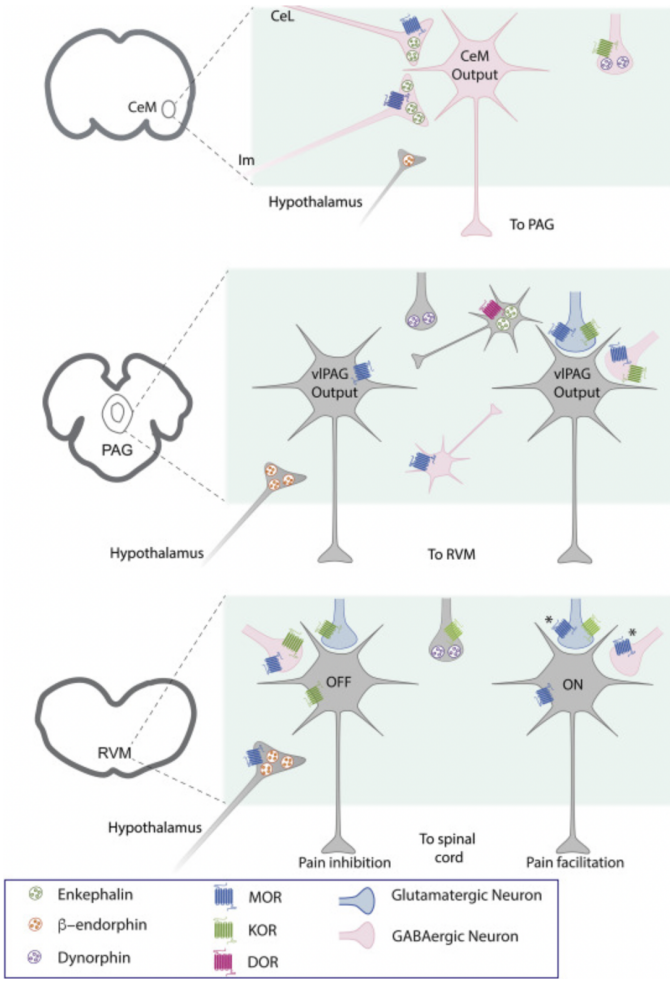

Figure 17. schematic depicting known endogenous opioids and opioid receptors in the amygdala (Bagely, 2020). *Muscular*

In an experiment investigating the potential inhibitory influences of ascending sensory pathways on the determination of central motor drive, investigators blocked somatosensory feedback during exercise using fentanyl, an opioid analgesic (Amann, 2009). This allowed investigators to selectively block activity in sensory pathways without interfering with motor nerve activity. Fentanyl increased the subjects tolerance for pain in the subjects lower extremities by binding to spinal opiate receptors and attenuating the activity of nociceptive, metaboreceptive, and C fibers which are thought to project into the primary somatosensory cortex of the brain (Amann, 2009). Researchers observed a rise in higher central motor drive, power output, and excessive peripheral fatigue suggesting that the sensory pathways inhibit supraspinal areas of the CNS and contribute to the determination of central motor drive (Amann, 2009). The results showed that peripheral feedback inhibits central motor output.

A reduction in central motor output is significant with respect to PAD as we can hypothesize it is a driver for the patient slipping into a deep sleep prior to a cessation in motor output and death.

Mitochondrial

Fentanyl administration also induced increases in locomotor muscle power and additionally exacerbated metabolic acidosis via hypoventilation and respiratory acidosis, causing a rightways shift in the oxyhemoglobin dissociation curve, reduction in alveolar partial pressure, and greater oxyhemoglobin desaturation (Amann, 2009). This caused an exaggeration

in arterial hypoxaemia, resulting in accumulation of inorganic phosphate, which together with the increase in H+ from acidosis and inorganic phosphate generated through hypoxaemia, accelerated fatigue (Amann, 2009).

With respect to PAD, arterial hypoxaemia is reduced oxygen saturation which can lead to death due to reduced oxygen supply to vital respiring organs responsible for sustaining life. Combined with a shift in the oxygen hemoglobin curve, the blood's affinity for oxygen will be reduced, exacerbating hypoxaemia, and resulting in death. Furthermore, fatigue will cause the patient to slip into sleep prior to death.

The Physiology of Drug Induced Death

Summary

Benzodiazepines

- Shortening of cardiac potentials results in induced inducibility in the form of tetanic contractions. Tetanic contractions of the heart impairs cardiac rhythm and the ability to pump blood to the body leading to cardiac arrest and death.
- A reduction in tidal volume may result in alveoli collapse, impairing gas exchange and preventing hemoglobin oxygen saturation resulting in a lack of oxygen to respiring cells (and therefore death). This is compounded by a decrease in the respiratory control ratio through ligand binding to PBR and resultant Ca+ influx in mitochondria.
- Benzodiazepines act as muscle relaxants, preventing side effects like vomiting during the dying process and increasing comfortability in the final moments of patients.

Barbiturates

- Block transport of calcium in excitable tissues preventing convulsions during death
- Depression of the nervous system, the chemical mechanisms of breathing, resulting in respiratory failure and death.
- Inhibit mitochondrial respiration, resulting in impairment of the functions of vital life-sustaining systems, resulting in death.

In PAD, barbiturates and benzodiazepines are typically administered together (**Table 1**)

Opioids

- Depression of cardiac contractility, preventing the circulation of oxygenated blood to respiring cells resulting in death.
- Non-cardiogenic pulmonary oedema resulting in respiratory failure and death.
- Analgesic effects and induction of sleep increasing comfortability of passing.
- Reduced oxygen saturation via shifting of oxygenation curve resulting in hypoxaemia and death

Knowing how these drugs induce physiological changes at the cellular level to induce death is important to understand if we are to attempt to legitimize their use. It is equally important, however, to consider how the consumption of PAD drugs acts on a grander scale. What do we observe with patients who opt for PAD at a psychological, or situational, level?

Case studies

It is important to think about these studies as it gives us a sense of what the situation is actually like for those undergoing the procedure, and loved ones who may be affected by their passing. It is vital that we consider these social, and value-based qualities in our evaluation of methods of life cessation as a large part of the perception of quality of life is determined by the accessibility and autonomy given to these. It is not enough then, to be blinded by the coming-and-goings of small-scale mechanisms and ignore psychological and environmental challenges which arise in human death as in the grand scheme of things, these are the areas which we truly observe and therefore truly feel and are moved by.

To refresh our memory in order to fully appreciate the differential death experience when using PAD drugs, I will again summarize case study one, the experience of death by forgoing artificial hydration and nutrition. Under these circumstances, individuals most often find themselves in hospitals or palliative care facilities. The reason for this is to manage unwanted symptoms associated with dehydration and starvation. In this way, patients are restricted to the bedside as they slowly meet death over a period of a few days. An individual has to *endure* their death rather than *experience* it. I ask you to look over case study 1 if this feels unfamiliar.

I will now outline two further case studies which concern death by PAD.

Case Study 2

The following case describes the perspective of a friend, overseeing the death of a patient (Tom) through PAD.

> Tom was diagnosed with metastatic cancer, feeling diminished at the prospects of the rest of his life, the patient opted for PAD. The process allowed him to plan out the rest of his life, paperwork, wills, books he had not yet read, dinners with friends. The patient decided on the day of his death according to the weather, as to not inconvenience his friends at the upcoming funeral. Together the friends emptied 100 capsules, dumping the powder into a whiskey glass. When the moment arrived at which Tom decided to end his life, he and his friend drank together one last time on the sofa of his living room.
>
> The friend then described the minutes following administration. He explained his fears, stating his worries that the death would be traumatic, characterized by vomiting or convulsing. He was relieved to find the physicians were right, and that Tom fell asleep quickly, slid into a coma, his breathing slowed and his heart stopped in two minutes.
>
> The friend recalled the experience as a fitting end to his friend's life, " it wasn't a religious ceremony, chanting, or singing, it was his dying process. A part of his life".

(Buchbinder 2018).

This case study does a brilliant job at breaking down the stigma that PAD is a form of suicide triggered by prospects of loneliness, or a desire to avoid dependency. Tom here, was utterly dependent upon his friend throughout his last moments. The process appears, contrary to what you would think, oddly intimate. In his final moments Tom did not lean into independence and become a recluse, but enlisted a friend to be entrusted with quite possibly the hardest

decision Tom had ever had to make. Tom needed support, and the structure of the PAD process allowed him to receive it in the comfort of his own home. It is strangely conclusive. Another flawed worry that this case articulates well is the risk of the permanence of the decision. People often fear that this is a decision made upon rash instincts, that like suicide these feelings come and go - they are unstable. It is clear that that is not the case here. Tom's death was regimented, organized, and well thought out even to the extent that part of his decision-making process was to not inconvenience others. There is no room for the doubt that this decision was one made with spontaneity. Every aspect one could think of considering in his personal life and beyond, Tom had already thought of, and produced a fitting end to. The phrase "fitting end" leads nicely into my next point. That Tom was able to characterize his death much like he characterized his life. Upon his terms.

Case Study 3

> Other deaths involve ritual elements, a final toast, or spiritual requirements due to the highly personalisable nature of PAD. Take for example that on Renee Long, a 60 y/o woman with a terminal cancer diagnosis. Her death was supported by her spiritual community, and aligned with her personal beliefs. The following is an account from a friend present at the time of death.
>
> A circle of close friends began a ritual around the drug, lighting candles and emptying capsules together. It was playful, loving, and had beautiful energy. They prepared it in a very sacred light way. Renee died lying in bed, surrounded by loved ones

> with her spiritual leader on one side and her brother on the other. During the ten minutes between Renee ingesting the medication and taking her last breaths, everyone was holding her, singing and praying. Renee embraced dependency in the months leading to her death, until the very end.

(Buchbinder 2018)

Our last case illustrates the moral and personal flexibility of PAD, a quality that can never be offered in starvation and dehydration. Many people are concerned that PAD is not consistent with firmly held ethical beliefs within a community. Renee's case highlights how this method can be tailored to fit the value and belief systems of almost any individual. Much like Tom's case, it too highlights the desire for community in death. How death can be incorporated into life in a celebratory way. It shows that the experience of death can be as much of a process of human existence as life itself. We can argue that individuals facing terminal illness should be allowed the option to cessate their lives in a way which is meaningful for themselves, in a way which unites their community, values, and history.

We can make some significant comparisons between the processes of dying between forgoing life sustaining treatment (Case study 1), and administration of PAD drugs (Case studies 2 and 3). The most obvious distinction between the alternative measures of death are that of the circumstances of death. Although in all instances, the decision to die is enacted by the patient at will, with regards to ultimate control over the dying process, it is clear that patients who opt for

PAD undergo a process which is more distinct. The extent of control for those forgoing sustenance is limited to merely making the decision to pursue death. Beyond this decision there is little control over the time frame or the experiences leading to death. Although in these circumstances it has been found that death is not uncomfortable, it is clear that there are a myriad of side effects which the patient must endure which persist for days leading until death. This type of experience is not the case for those who opt for PAD. Thus in the decision making-process, physicians should consider which symptoms are most appropriate for the patient to ensure they can endure the consequences of their decision and that it aligns with their values and wants. Similarly, decisions regarding which treatment plan is appropriate should be decided by the patient on the basis of their needs. If an individual requests immediate cessation of life, PAD administration offers the most appropriate solution. However, patients who prefer a more natural, prolonged process of dying may prefer to opt for the method which forgoes life-sustaining measures.

End of Life Decision-making

In an investigation determining whether there was a difference in the quality of the dying experience from the perspective of family members, researchers conducted a cross-sectional survey (Quality of death and dying questionnaire) of family members of 149 individuals (Smith, 2011). 52 individuals opted for lethal prescriptions, 34 requested but did not receive lethal prescriptions, and 64 did not pursue PAD. The results indicated that those receiving PAD

prescriptions had higher quality ratings on items measuring symptom control and preparedness for death in comparison to those who were denied, or did not request PAD (Smith, 2011). Few differences were noted in sections which pertained to connectedness, transcendence, and overall quality of death (Smith, 2011). These results suggest that PAD is not a worse option than forgoing life sustaining treatment, and in some cases, is in fact rated as a better death-experience by family members.

PAD is preferable over alternatives like forgoing life sustaining treatment not only because of the greater control over one's life timeline, but due to the greater control over one's self. By this I mean that PAD allows patients to avoid the slow decline of terminal illness which would render them unrecognizable to the person they once were (Buchbinder, 2018). This allows patients to avoid the circumstances under which a death of self may occur before their biological, physiological death arrives. The value systems of individuals opting for treatment then, is integral in the decisions they make when approaching death, just as it is in the decisions they opt for during life. Take for example, an individual characterized by their position as a public speaker figure, where their ability to communicate is obviously fundamental to their idea of self. If the future of their disease threatens this ability, it does not simply threaten an arbitrary bodily function, but also threatens one's personhood. To this patient then, the prospect of death may be preferable over waiting for themselves to deteriorate, losing themselves in the process.

Ethics

Patient autonomy in a healthcare setting, describes when a patient is able to determine which medical interventions they would like to consent to, and those they would like to refrain from. This ideology leads to the development of informed consent, a process by which a patient is only able to make decisions if they are provided with all the relevant information regarding the consequences of intervention and non-intervention (Dugdale, 2019). Patients will then be capable of possessing the agency that allows them to make informed decisions regarding the outcomes and consequences of their choices. The existence of such a procedure extends to PAD, in that if patients are considered capable of possessing the authority to make medical decisions in life, some of which may carry risks of death themselves, then patients should possess the same abilities when it comes to the circumstances of their death. Indeed, if a physician is to argue that the core of the profession is to relieve patients from suffering from illness and disease, PAD is consistent with this ideology. Thus, relieving potential suffering through PAD drug administration may be considered to be meeting the goal of acting humanely and compassionately. In the event that the individual, bound by suicidal ideation, reaches a barrier in accessing PAD, they may seek to employ alternate measures to reach their goal of death. This may result in patients seeking alternative, unsafe, ineffective, violent measures, a worst case outcome for patients and families.

The major conflict in comparing starvation and dehydration with PAD occurs at the level of ethics. Many individuals feel as though there is a significant difference between the procedure of "letting someone die", that is allowing them to refuse treatment, and "being engaged with the

dying process", or providing them with the means. Those who present voluntary starvation and dehydration as an alternative treatment option for end-of-life suffering that avoids the moral ambiguity associated with PAD would be incorrect. Take for example, patient A who refuses artificial fluids and nutrition on the basis of an evaluation of the benefits and drawbacks of continued sustenance, versus patient B who forgoes these treatments as a means to end suffering (Jansen, 2015). Patient A, is not making the decision out of duress, it is an informed, rational, chosen decision. Patient B however, is committing suicide as their primary motivator is to escape pain. Thus, there is no significant difference between offering forgoing life-sustaining artificial nutrition and hydration, and offering PAD-drugs as end-of-life palliative care treatment options. Furthermore, the problem of allowing an individual to decline the medical consequence of artificial hydration and sustenance is only met given that the patient has found themselves in a circumstance under which they require sustenance. This in itself appears as though it would fulfill the criteria of suicide, not of rejecting medical treatment.

Conclusion

To synthesize, this paper covered a review of PAD, a description of the alternatives, an investigation into the major drug classes used in PAD and their physiological action including how they induce death, and an examination of case studies. It was important to present not only

how PAD drugs cause death from a cellular mechanism and network perspective, but from that of a grander experiential and psychological level. This is because it is not only necessary that physicians, who are capable of understanding biological jargon and methods of pharmaceutical action, can understand the mechanics of PAD induced death, but also the individuals who are administering the drugs and their loved ones. This is because the information provided by the larger-picture subjects are tangible to all. For the uneducated, no amount of information about the movement of ions or changes in protein configuration will help them understand which method of death is right for them or their loved ones. What will matter, however, is what the dying process can look like on a physical level, how to know whether their decision is the right one, whether it is ethical. This thesis then, provides a plethora of information which is accessible to all, regardless of education status. It will help to bridge the gap between professionals and the individuals they serve by accounting for many complications and questions regarding the PAD process.

Investigations into the physiology of drug induced death of opioids, benzodiazepines, and barbiturates, has shown that this mode of death is non-traumatic, it occurs quickly and often without adverse effects. In contrast with that of forgoing artificial hydration and nutrition, this process is faster and offers less ambiguity. As one of the major fears of individuals with terminal illness who choose to die has been shown to be 'lack of control', PAD appears to be the obvious choice to avoid this circumstance whereas forgoing life sustaining treatment simply exacerbates this process. Other physiological changes which occur during administration of PAD drugs contribute to the ease of life into death, such as their anticonvulsant, anti-nauseating, and

muscular relaxant properties. In order to ease the process of dying in starvations and dehydration, individuals must be administered drugs to cope with physiological changes, often these drugs, particularly opiates which are administered as analgesics, increase the speed of dying.

This is important information to contemplate due to the disconnect between PAD as an option for humane end of life care, and the legislation globally which prevents PAD from being enacted for those suffering with terminal illness. This is a failure on behalf of governing agencies worldwide to promote patient agency over their value systems and an impediment to patient autonomy regarding medical decision making. It reinforces the ideology that any life is superior to none at all (regardless of quality of life) and reinstates physicians in a position of academic superiority over the individuals they serve which results in outcomes that do not coincide with the wishes of their patients. By not considering incorporating PAD as a treatment option for the terminally ill, following the same informed consent laws as other procedures, governing agencies are preventing individuals from exercising their rights as a person, and therefore are infringing upon their personhood. This sets a dangerous precedent as it brings into question how else this refusal of personhood can be manipulated and infringed upon for terminally ill individuals at the end-of-life. If we are to truly value the free will of individuals, medical professionals should amplify their voices. This is especially pertinent in situations like those emphasized in this paper where patients are facing their own morality. In order to promote human freedoms in later moments of life, where often all else is uncontrollable, it is a necessity to offer PAD as an option. This requires that physicians are educated of the realities of the procedure, and biases or misconceptions regarding the ethics of collaboration with patients during PAD are overcome. To

not pursue this route is to force individuals to die unpleasant deaths under circumstances they may not want due to a lack of alternatives.

There exists the opportunity for future research into the ethical considerations regarding who should be eligible for PAD outside of those with terminal illness. Special considerations must be made to account for low income individuals, those with a history of mental illness, and classes of individuals who are systematically oppressed such as those with disabilities. This research should be inclusive of societal biases around sensitive groups to ensure that misconceptions do not shape legislation. Special attention should be paid to implications of offering PAD procedures under insurance policies. There is an investigation opportunity here to determine whether societal biases regarding the quality of life of groups of vulnerable individuals will result in PAD being offered at higher rates than for the general population. We need to ensure that insurance companies are not allowing for PAD under malicious intent, e.g that vulnerable people feel pressured to opt for PAD over non-futile treatment due to financial benefits to the insurance company and/or family members. This is an important area of research in order to preserve the well-being of all vulnerable members of society and ensure they are being offered the same protections as phenotypical, able-bodied and able-minded individuals. Without sociological and economic considerations to determine the ethics of offering PAD to the larger population, this thesis can only serve as an informative review of opportunity for terminally ill patients.

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