Analgesics and their mechanisms of action

Since ancient times, countless medicinal compounds have been used for their analgesic, or painkilling, effects. Usually, these compounds are derived from plants, which do not produce them for their analgesic effects on humans but for protection against pathogens and predators. For example, the ancient Sumerians and Egyptians used willow bark as an analgesic over 3500 years ago (Desborough and Keeling, 2017). It was discovered much later that the active agent in willow bark responsible for its analgesic effects is salicin (Ding and Ding, 2020). Over the years, a multitude of other analgesics have been discovered, refined, and mass-produced for use by hospitals and the public; some have even become household medicines that are now used daily. However, despite their widespread use and huge role in pain relief, it is still unclear how some types of analgesic truly work.

In an interesting interview with Professor Donald Cairns, we discussed the importance and benefits of pain. Most of the time, pain serves as a warning of danger from the body that causes you to act against it, such as when touching a hot stove. In these situations, pain is important to stop further damage from happening and to alert the individual about the damage done. However, in cases where the pain does not serve a useful purpose or is problematic (such as the burning sensation after already having touched the stove and treated your hand), analgesics can be helpful in stopping inconvenient pain.

Generally, analgesics work by either blocking pain signals sent to the central nervous system (CNS), or by directly affecting the CNS to reduce the perceived intensity of pain. There are two main types of pain that analgesics are used to relieve: nociceptive and neuropathic pain.

Nociceptive pain is caused by a physical stimulus, like being pricked by a needle or pain from a kidney stone. It can be somatic (felt in superficial areas of the body like the skin and muscles) or visceral (felt in the internal organs) (Jacques, 2023). There are four main stages that lead to the pain being felt. The first stage is transduction, where the injury triggers the release of chemicals such as prostaglandins which cause nociceptors (receptors that detect painful stimuli) to generate an action potential, or an electrical impulse (O'Donnell, 2012). The action potential travels to the brain along neurones, travelling across synapses (the space between

neurones) by the diffusion of neurotransmitters which bind to the next neurone – this stage is transmission. At the perception stage, the individual becomes aware of the pain once the signal reaches the brain. The last stage is modulation, which is when the brain alters and even suppresses the pain (O'Donnell, 2012) by releasing different chemicals.

Neuropathic pain is caused by a damaged nervous system and commonly causes chronic pain (Tidy, 2020). For example, damage to the thalamus (a pain-modulating region of the brain) can cause a rare condition called Dejerine-Roussy syndrome (Jahngir and Qureshi, 2023). Patients with this condition often experience allodynia (pain from a usually non-painful stimulus, like air blowing on the skin) and hyperalgesia (intense pain from a stimulus that usually causes less pain) (Jensen and Finnerup, 2014). Nociceptive and neuropathic pain have different causes; therefore they must be treated with different types of analgesics.

Non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat nociceptive pain and most of the time, they specifically act on the transduction stage (Jacques, 2023) by targeting cyclooxygenase (COX) enzymes, which are responsible for inflammation and sensitivity to pain.

COX enzymes metabolise a fatty acid called arachidonic acid (ARA) into different metabolites (Ricciotti and FitzGerald, 2010) which is shown in Figure 1. Each COX enzyme is made of two monomers, which each have a COX site and a POX site, meaning there are two COX sites in total (Dong *et al.*, 2011). When a fatty acid binds to one of the COX sites, that monomer becomes an allosteric monomer (a monomer that causes a conformational change in the shape of the enzyme) and makes the second monomer become catalytic (able to speed up the main reaction). ARA then binds to the catalytic monomer and a tyrosyl radical (a tyrosyl molecule with an unpaired electron) removes a hydrogen atom from it. Two oxygen molecules are added to it, turning the ARA into prostaglandin G_2 (PGG_2) (Yuan *et al.*, 2009). The PGG_2 then binds to a POX active site, where it is reduced (gains electrons) to make prostaglandin H_2 (PGH_2) (Suñer-Rubio *et al.*, 2020).

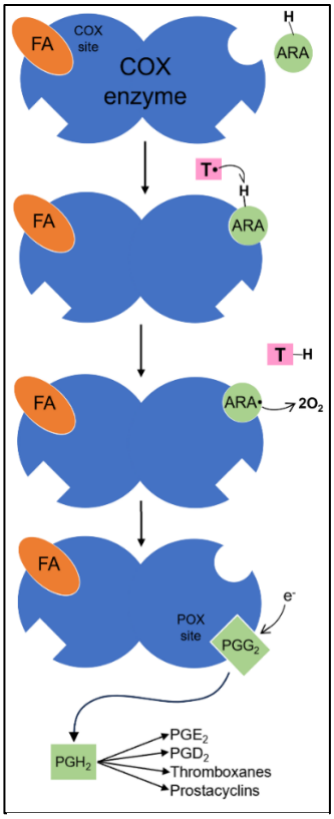

Figure 1 - A model of the action of COX enzymes, where FA represents a fatty acid and T• represents a tyrosyl radical. (self-made)

Various enzymes can then catalyse the PGH_2 into different metabolites including PGE_2 and PGD_2 (Taylor-Clark *et al.*, 2008). These prostaglandins are responsible for generating the action potential that transmits pain signals in transduction, for vasodilation (which causes inflammation), and for making neurones more excitable (i.e. more able to generate an action potential), therefore increasing sensitivity to pain (Dharmshaktu, Tayal and Kalra, 2012). PGH_2 can also be catalysed into thromboxanes which have an important role in platelet aggregation during blood clotting (Ghlichloo and Gerriets, 2023).

There are two types of COX enzymes, COX-1 and COX-2. COX-1 can be found in most body tissues and is also important for kidney function and protecting the stomach lining from digestive juices (Eustice, 2022), while COX-2 is found in inflammatory cells and is the type of COX enzyme that causes most of the unwanted inflammation and pain (Vane, Bakhle and Botting, 1998).

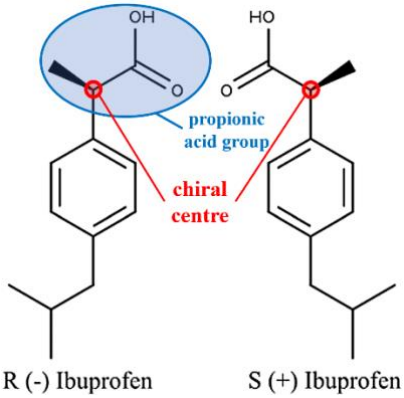

Figure 2 - The enantiomers of ibuprofen. (modified from Freiberger, 2021)

Ibuprofen and aspirin are two examples of common NSAIDs. Ibuprofen is a propionic acid derivative (Ghlichloo and Gerriets, 2023) and a competitive inhibitor of COX enzymes (Ricciotti and FitzGerald, 2010), meaning it can block the active site of the enzyme and stop ARA from binding to it and being metabolised. This stops prostaglandins from being synthesised which prevents inflammation and the action potential from being generated in transduction, reducing the intensity of pain. Interestingly, ibuprofen has two enantiomers (optical isomers that are mirror images of each other, like your left and right hand) due to its chiral centre in the propionic acid group (Rainsford, 2003), as shown in Figure 2**.** While there have been cases of drugs where the enantiomer had severe side effects such as the birth defects caused by the enantiomer of (R)-thalidomide (Tokunaga *et al.*, 2018), there have been no cases of side effects caused by the ibuprofen enantiomer (Rainsford, 2003).

Unlike ibuprofen, aspirin is an acetylated salicylate (Ghlichloo and Gerriets, 2023) and is a more aggressive NSAID as it can irreversibly change the shape of a COX enzyme's active site and stops the enzyme from functioning permanently (Vane and Botting, 2003), therefore

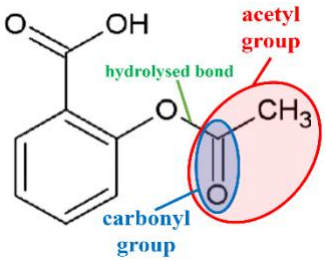

Figure 3 - The skeletal formula of aspirin, showing the acetyl group. (modified from Gawad, 2013)

permanently stopping that enzyme from synthesising prostaglandins. As discussed with Professor Donald Cairns, this happens when an OH group on an amino acid (the monomer that proteins and enzymes are made from) in the COX enzyme attaches to the carbonyl group shown in Figure 3. The bond between the carbonyl group and the oxygen is hydrolysed (broken), which leaves the acetyl group, also shown in Figure 3, attached to the COX enzyme. This causes the irreversible change in the shape of the enzyme's active site.

Aspirin and ibuprofen are both non-selective NSAIDs that target both COX-1 and COX-2 (Eustice, 2022), meaning that although the inflammation and pain caused by COX-2 is subdued, the beneficial functions of COX-1 are also inhibited. For this reason, non-selective NSAIDs, particularly aspirin, are not recommended for those who have a history of stomach ulcers or kidney disease (NHS, 2021).

In the interview, we discussed NSAIDs that are COX-2-selective, such as celecoxib – these drugs are named coxibs (Dong *et al.*, 2011). They mainly inhibit COX-2 by targeting a small indent in the active site of COX-2 that does not exist in COX-1 (Hawkey, 2001). Since they inhibit COX-1 less, they also cause fewer side-effects related to COX-1 inhibition than non-selective NSAIDs (Eustice, 2022). However, very few coxibs are available for use as most coxibs discovered so far cause other very harmful side effects that outweigh the benefits, like increased risk of cardiovascular disease (Hawkey, 2001). The use of the few available coxibs is limited as they could potentially have similar side effects as well. Professor Cairns suggested that the side effects may be caused by COX inhibition elsewhere in the body, since coxibs are very potent COX-2 inhibitors. This could affect cardiovascular function and cause side effects.

Paracetamol is another analgesic that decreases nociceptive pain. It is considered in a class of its own since its mechanism of action is still unclear, and it does not behave exactly like NSAIDs or other types of analgesics. There are, however, two leading ideas about how it works to relieve pain.

One idea is that paracetamol reduces pain when it inhibits COX enzymes by reducing (adding electrons to) the enzyme through an interaction on the POX site (Smith, 2009), affecting the shape of the active site similarly to some NSAIDs - this only happens when there is a low concentration of ARA. Under this condition, COX-2 is the main form of COX (Ghanem *et al.*, 2016), so paracetamol mainly inhibits COX-2. Paracetamol also does not reduce inflammation as NSAIDs do because inflammation causes high concentrations of peroxides, which makes paracetamol a less effective reducing agent (Ghanem *et al.*, 2016).

An alternative mechanism of action is based on the activity of one of paracetamol's metabolites, N-arachidonoylphenolamine (AM404), which is formed when a paracetamol molecule loses an acetyl group and then binds to ARA (Ghanem *et al.*, 2016). Figure 4 shows that this metabolite has a very similar structure to a neurotransmitter called anandamide (ANA), which can bind to cannabinoid (CB) receptors found in the CNS and decrease nociceptive pain by affecting pain modulation (Donvito *et al.*, 2018). It is believed that AM404 can activate the CB receptors to some extent and also reduce the uptake and removal of ANA by inhibiting transporters, which causes ANA to remain in the CNS and bind to CB receptors (Ghanem *et al.*, 2016), causing it to decrease nociceptive pain.

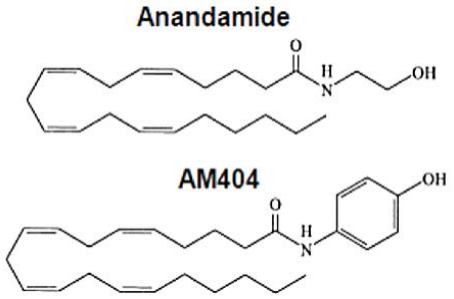

Figure 4 - The skeletal formulae of ANA and AM404. (Ghanem et al., 2016)

Although paracetamol also has an acetyl group like aspirin shown in Figure 5, it cannot work in the same way by donating an acetyl group. Professor Cairns explained that this was because the acetyl group in paracetamol is bonded to an amine group, which makes it a much more stable bond. The stability of the bond means that the acetyl group does not hydrolyse easily, so it does not attach to the COX enzyme.

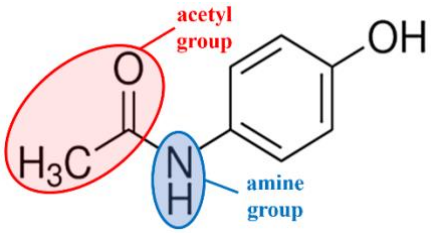

Figure 5 – The skeletal formula of paracetamol showing the acetyl and amine groups. (modified from Muthuselvi, 2016)

While paracetamol most likely works through both suggested mechanisms, recent evidence suggests that the action of its metabolite AM404 may contribute more to its analgesic effects. Sharma *et al.* (2017) found high concentrations of paracetamol and its metabolites in the

spinal cord fluid soon after intravenous injection of the drug which suggests that paracetamol acts mostly in the CNS rather than on COX enzymes around inflammatory cells.

Analgesics that target neuropathic pain, such as opioids, have very different mechanisms of action since the cause of pain is the misfiring of damaged neurones, rather than the action of an enzyme and its product. Most opioids directly affect calcium channels, which are important for controlling the entry of Ca^{2+} ions into synaptic terminals (the area of a neurone near the synapse) and controlling neurotransmitter release at synapses (Szucs-Reed and Gallagher, 2013). Opioids can affect these channels and inhibit the release of Ca^{2+} ions (Chahl, 1996) which in turn stops the release of neurotransmitters that would have otherwise passed on the pain signal to the brain.

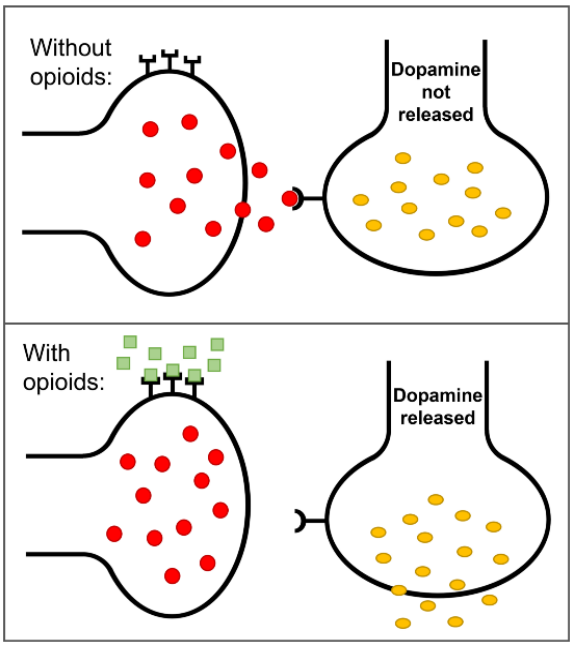

Figure 6 - A model of inhibitory neurones (left) and dopamine (right) functioning with and without opioids. Inhibitory neurotransmitters are represented by red circles, dopamine is represented by yellow ovals, and opioids are represented by green squares. (self-made)

Another way opioids can relieve neuropathic pain is by binding to the mu opioid receptor (MOR) found on inhibitory neurones in the CNS, particularly in the thalamus (RxList, 2007). When functioning normally without opioids, inhibitory neurones release neurotransmitters that prevent the secretion of dopamine. However, when opioids are bound to the MORs, the inhibitory neurones are essentially disabled, and the secretion of dopamine can occur as shown in Figure 6 (Osmosis, no date). When dopamine is released in pain-modulating areas of the brain like the thalamus, it reduces the perceived intensity of pain.

Furthermore, dopamine can produce a feeling of euphoria which causes an individual to feel unconcerned with the pain, despite them still feeling some of the pain (Chartoff and Connery, 2014). This emotional high is one of the most significant causes of opioid addiction and is the reason why opioids are usually not prescribed until necessary in order to prevent misuse (Osmosis, no date). They are also not prescribed for long periods due to the risk of opioid

tolerance, which is where the opioid receptors become less sensitive to the drug and the patient would therefore need a higher dose for the same pain-killing effect (Raehal *et al.*, 2011).

Despite the risks of taking opioids, they are still prescribed at times. For instance, morphine is commonly given to ease pain after major surgeries and is a very strong opioid. It works like most other opioids and has a high affinity for (very easily binds to) MORs, making it very effective at reducing pain. Hydrocodone is another opioid that is commonly prescribed for minor pain that is unresponsive to other analgesics (Fookes, 2022). It has a lower affinity for MORs compared to morphine, but hydrocodone works slightly differently as it does not directly bind to the receptor itself. Instead, it is metabolised in the liver into hydromorphone which has a much higher affinity to MORs than hydrocodone and has stronger analgesic effects (Szucs-Reed and Gallagher, 2013).

Pain is known to be subjective and specific to the individual. This is shown by the existence of the modulation stage of nociceptive pain which suggests that the brain can manipulate the pain experience. For instance, stress-induced analgesia is triggered during a frightening or stressful event and makes the brain suppress the pain perceived, despite the pain still being present (Butler and Finn, 2009). The brain may also act in a similar way after analgesics are taken due to the placebo effect.

Vase *et al*. (2009) compiled a variety of studies that tested the placebo effect of analgesics. In each study, volunteers were given a placebo agent and were told that it was a powerful analgesic. The volunteers were exposed to a pain stimulus before and after taking the placebo agent, and the pain intensity was recorded. Although it is difficult to quantify a variable as abstract as the intensity of pain, most volunteers said that they felt less pain after taking the placebo agent. Since there was no analgesic agent in the placebo, the decrease in the perceived intensity of pain may have been due to the volunteers expecting that they would feel less pain as they believed they had taken a painkiller. This suggests that as well as the biochemical changes caused by analgesics, perhaps some of their painkilling effect comes from simply consuming the drug and the user being convinced that their pain would soon be lessened.

The extent of the placebo effect in analgesics as well as other drugs was also discussed in the interview, where Professor Cairns described how drug trials must be designed in such a way that the placebo effect is eliminated due to its very real effects, especially with analgesics. For instance, they are normally double-blind (i.e. neither the volunteers nor the scientists know if the drug they take or administer is a placebo or not), which prevents bias from both parties.

As shown by the numerous examples of painkillers, analgesics are powerful compounds that have increased the quality of life for many people, especially those living with chronic pain. Although the mechanisms of action of some types are not clear yet, it is almost certain that science will eventually advance to a level that will allow us to understand how they work, and perhaps even discover new drugs that can influence the human body, treat chronic conditions like Dejerine-Roussy syndrome, and more effectively control pain.

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