The sensation of pain is subject not only to modulation during its ascending transmission from the periphery to the cortex \[75\] but also to segmental modulation and descending control from higher centres. This control is manifested via pathways that originate at the level of the cortex, the thalamus and the brainstem (the periaqueductal grey: PAG, raphe nuclei and locus coeruleus subcoeruleus complex: LC/SC). Descending inhibition from cortex and thalamus is, in part, mediated via relay stations in the brainstem. The main neurotransmitters implicated in descending pain control are serotonin (5-HT), noradrenaline (NA) and the endogenous opioids, although others also play a role.

Methodological considerations

It is important to realize that most of our current understanding of descending inhibition is based on results from three kinds of experiment:

1. Behavioural testing has shown that electrical stimulation of individual brainstem nuclei can be analgesic in awake animals.

2. The same (or similar) stimulation conditions that produce analgesia in awake animals can, in anaesthetized animals, inhibit cells responding to noxious inputs in the dorsal horn of the spinal cord.

3. Lesions of various nuclei and microinjections of drugs into them have provided evidence of the involvement of these nuclei and their transmitters in the descending control of nociception.

Several factors need to be considered in the interpretation of data from these three different types of experiment. Some of the most important of these factors are listed below.

ANAESTHETIC STATUS OF THE ANIMAL

It is intuitively obvious that anaesthesia is a potential problem in any system where modulation of sensation is the variable under investigation. Anaesthesia is essentially a composite phenomenon consisting of analgesia, amnesia and akinesia. It is clear that it is difficult to study the normal physiological mechanisms of pain under such circumstances. At best, pain responses will be only quantitatively altered by anaesthesia. However, this is not always the case: it has been shown, for instance, that anaesthesia can not only depress cerebral metabolic activity but also, in some nuclei, reverse the direction of response to drugs \[40\]. Behavioural experiments of the tail-flick type offer the best way round the problem of anaesthetic modulation. However, other problems arise with these experiments (see below).

The spinal cord moves a considerable amount relative to the bony vertebrae in a freely moving animal. It is thus tricky to examine single cell responses in the spinal cord of such an animal. Thus the vast majority of such studies have been performed in immobilized anaesthetized preparations. Nevertheless, differences in the depth of anaesthesia and type of anaesthetic used have often been touted as explanations for variable or inconsistent results between laboratories in the proportions of cell types detected (multireceptive \textit{vs} noxious-specific, for instance) and their response to different peripheral stimuli \[73, 85\].

There is little doubt that tonic supraspinal modulation of nociceptive neurones \textit{is} operative in the anaesthetized cat since cooling of the cord rostral to the recording sites enhances responses to noxious stimuli \[125\]. However, multireceptive neurones are less common in the awake than in the barbiturate-anaesthetized cat \[24\] suggesting that descending inhibition may, to some degree, be masked by general anaesthesia.

Slightly more disquieting is the possibility that components of the pharmacology of descending inhibition may be differentially modified by anaesthesia. For instance, morphine may \textit{increase} LC neurone firing in conscious cats while \textit{decreasing} activity under chloral hydrate anaesthesia \[94\].

DEFINITION OF A “NOCICEPTIVE” NEURONE

Many neurones within the spinal cord respond to noxious stimuli. There are functionally two main groups of dorsal horn cells: “nociceptive-specific” and “multireceptive” (also called “wide dynamic range” (WDR) or “convergent”). The former respond solely to noxious stimuli while the latter are also activated by innocuous stimuli such as touch. Even WDR is an uncertain definition since some may respond preferentially or equally to innocuous or noxious stimuli. That is not to say, however, that these cells are necessarily involved in the sensation of
pain. Nociceptive responses include not only those as a result of a pain sensation but also reflexes. For example, flexor motor neurones may respond, even selectively, to noxious stimuli yet presumably do not contribute to the sensation of pain [129].

It is difficult to draw a causal relation between the activation or inhibition of a spinal cell that responds to noxious stimuli and the perception of pain unless it can be shown that the cell is part of a sensory pathway such as the spinthalamic or spino-reticular tracts. Nociceptive interneurones are a particular problem in this respect unless their position within the synaptology can be determined. It is really only when defined cells are recorded, such as by antidromic activation from the thalamus [14], that conclusions may be drawn with confidence about their relation to pain.

Iggo and colleagues [50] raised an important question over the population of cells sampled in electrophysiological experiments. For instance, although there are hundreds of thousands of cells in each millimetre of cord, often only a handful may be recorded in a typical experiment. There is, furthermore, the risk that “even if a group of spinal neurones could be defined as important in transmitting impulses which are important in the perception of pain, these may not be the appropriate ones for studying a particular control” [31].

It is relatively rare for electrophysiological responses to be correlated with behavioural pain responses. Even when this has been done, large populations of cells cannot be sampled and surprising dissociations can result. For instance, both electrical stimulation of nucleus raphe magnus (NRM) and microinjection of morphine into the nucleus are antinociceptive in the tail-flick test. However, NRM stimulation inhibits the response of dorsal horn neurones to noxious stimulation, while morphine does the opposite [67].

INTERPRETATION OF BEHAVIOURAL DATA

Behavioural measures of analgesia are fraught with interpretational pitfalls. The two most commonly used algesiometric tests are the hot-plate and tail-flick methods. In the hot-plate test, animals (usually rats or mice) are typically placed on the surface of a metal plate held at ~ 50–55°C. The latency to lick the forepaw or hindpaw is measured. The tail-flick test is similar. A radiant heat source is focused onto a rat’s tail about 2–5 cm from the tip. The time until the rat withdraws its tail from the heat source is measured. Variants of the tail-flick test (where the heat is directed toward the snout) have been used in other species [18]. In each test the intensity of the stimulus is adjusted such that latencies are typically around the 5 s mark. Lower values are problematic because they approach the minimum response time possible and thus do not allow for the observation of hyperalgesic actions.

There are a number of considerations in behavioural tests. First, although the hot-plate test is felt to be supraspinally integrated [115], the tail-flick test is purely a spinal reflex. Although nociceptive, neither test differentiates between an action due to the sensory event and the motor response. In other words, an animal may fail to respond because it has suffered motor impairment, not because it has lost its sense of pain. This is a particular problem with stimulation in areas like the medullary lateral reticular nucleus that are involved in integration of sensorimotor information [53]. Second, both tests are potentially damaging in their own right and there is the risk, with repeated measurements, that local inflammation can alter pain thresholds. This can be avoided if single trials are used with a long (e.g. 24 h) separation [122].

Drug effects on response latency need to be interpreted with caution. Since the tests depend upon a competent motor response it is possible, for non-specific drugs to exert an apparent analgesic action solely because they have impaired motor function. It is thus highly advisable to test motor performance in parallel—by negative geotaxis, righting reflex [52] or, most sensitively, the accelerating rotorod test [88]. Despite these concerns, motor controls are all too infrequently used. However, without these essential controls, the interpretation of any behavioural data is suspect.

A further consideration, particularly applicable to many noradrenergic drugs, is that the agents may interfere with local heat transfer in the tail by vasodilatation or vasoconstriction [31]. Local vasoconstriction or hyperthermia, such as seen with alpha2 agonists [87] can also manifest itself as analgesia in thermal tests. Sedation too can interfere with supraspinally-mediated pain responses.

It has, nevertheless, recently been shown that behavioural measures provide relatively good correlation with c-fos activation in the dorsal horn [39] and that supraspinal opioids decrease both in parallel. Lesions of the dorsolateral funiculus block c-fos expression by noxious stimuli [133].

MICROINJECTIONS AND IONOPHORESIS

Microinjections or ionophoresis of drugs at specific sites have been used extensively to provide evidence that certain nuclei are or are not involved in descending control. As with all attempted localized applications of drugs, the primary concern is that the site of effect is spatially constrained. There is little point in using any means of local drug application (ionophoresis, pressure ejection, microinjection) if the drug reaches distant sites. The spread depends on the drug and the volume and concentration applied. For instance, it has been shown that diffusion of catecholamines and 5-HT is limited by interaction with uptake carriers and other brain constituents [100, 101, 112]. This is likely to apply generally to all natural neurotransmitter molecules applied into their natural neuronal environment (i.e., in regions which possess the appropriate innervation). 5-HT for example may be expected to diffuse further in areas devoid of serotonergic innervation and thus have more distant actions. Although the diffusion of exogenous drugs is likely to be less susceptible to modification by uptake carriers, interactions with receptors or uptake sites may occur and can alter the distribution profile.
The distribution of even small volumes can be surprisingly extensive in the brain. Bouhassira and colleagues [13] obtained a lesion of the rat PAG that extended 2 mm rostrocaudally with the bilateral injection of 4 g of ibotenic acid in 0.5 l of saline. Injection of as little as 100 nl of pontamine sky blue results in a dye mark up to 1.5 mm in diameter in the rat PAG [105].

When drugs are applied by microinjection a problem arises because the drug concentrations used are often sufficiently high for non-specific effects to occur, even accounting for diffusion and dilution of the compound. With ionophoresis, the concentration reaching the site of effect is even less easy to control although electrochemical detection allows a degree of quantification [5]. For example, ionophoretically applied naloxone (5 nA eject current) can sometimes block the effects of gamma-aminobutyric acid (GABA) on caudal reticular neurones [46]. A further consideration with any local application of drugs is the relation of the ejection site to synaptic geometry. Whereas the endogenous transmitter will stimulate the relation of the ejection site to synaptic geometry.

Consideration with any local application of drugs is the extent to which current spreads around the stimulating electrode [93]. This can be determined by several factors: electrode type (mono- or bipolar), the polarity of the stimulation (anodal or cathodal), pulse width and, in the case of bipolar electrodes, the orientation of the poles. For example, it has been shown that high currents (and 500 A is not unprecedented in stimulation analgesia) can, with 200 s pulse widths, stimulate cell bodies up to ~1 mm distant [93]. Currents this high thus have extremely limited spatial resolution. This is of particular relevance in nuclei such as the PAG where different types of antinociception (opioid and non-opioid) are mediated via anatomically distinct but adjacent subregions [8]. If very high stimulation levels are required to manifest analgesia in a particular nucleus this probably indicates that analgesia is not due to that nucleus but to more distant structures.

Conversely, many sites can produce analgesia with thresholds of only 25–50 A [56] suggesting that the source is adjacent. When accurately positioned, electrical stimulation can exert profound effects with relatively modest numbers of cells activated. For instance, it has been estimated that stimulation of as few as 30 neurones in the NRM can abolish the tail-flick response [45].

It is possible to exert anodal block of transmission in tissue immediately surrounding the electrode when pulse stimuli are used. This may explain circumstances in which high- and low-current stimulation exert opposite actions, as in the thalamic parafascicular nucleus (above). A knowledge of the electrical properties of the neurones (their chronaxie) is desirable.

There is also the consideration, particularly in nuclei with known spinal afferents, that stimulation-induced inhibition of nociceptive cells may be the result of antidromic activation. This can be discounted using collision-inactivation tests.

A further striking feature of stimulation-induced analgesia, in humans at least, is its longevity. Two or three 10-min periods of stimulation of the PAG are counted using collision-inactivation tests.

ELECTRICAL STIMULATION VARIABLES

From the very first observations that electrical stimulation of the PAG evokes analgesia [99], this tool has been central to research on descending inhibition. Indeed, it is striking how many sites within the brainstem (and above) seem to mediate the response: stimulation of the LC/SC complex, nucleus reticularis lateralis and magnocellularis, raphe dorsalis and nucleus magnus have all been shown to inhibit nociceptive dorsal horn cells. More rostral sites such as the thalamus and hypothalamus may also be effective (for review see Willis [129]).

The central question with brain stimulation is the issue of which elements are being stimulated. Electrical stimulation may act on cell bodies, dendrites or axons passing through the area of stimulation. Thus, it is possible that some of the “nuclei” that support stimulation-induced analgesia may do so simply because axons of passage have been activated.

Electrical stimulation of a given nucleus may not activate all elements equally. Thus, if two elements in the nucleus have opposing actions the net response could suggest that the nucleus had no role in descending inhibition. Alternatively, it may happen that low- and high-intensity stimulation will activate subsets of different elements preferentially [38]. For instance, stimulation of the thalamic parafascicular nucleus in humans causes either pain [72] or antinociception [4] depending on the stimulus intensity.

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SPECIES AND STRAIN VARIATIONS

There is a natural desire to extrapolate results from a single species to others, particularly humans. However, in the modulation of pain, there is evidence of major differences between species and even strains.

Although it is generally accepted that the locus coeruleus (LC) sends a major noradrenergic projection to the spinal cord in the rat [64], there is evidence that the Kolliker Füse (KF) nucleus is the primary source of descending noradrenergic fibres in the cat [113]. Stimulation of the LC/SC complex in the cat does inhibit the response of spinal neurones to noxious and innocuous stimuli. However, this inhibition is unaffected by prior reserpine or 6-hydroxydopamine lesions that lower tissue NA to <10 % of normal [47].

There may be differences in strains of a single species: tail-flick response latencies are faster in...
Wistar Kyoto rats than Sprague Dawley rats [74]. One cannot even assume that the same strains from different suppliers are equivalent: electrical stimulation of the LC is analgesic in both Harlan and Sasco strains of Sprague Dawley rats. However, antinociception in Harlan rats can be blocked by intrathecal yohimbine while the drug is ineffective in Sasco rats [127].

The pathway from NRM to the cord descends via the contralateral dorsolateral funiculus (DLF) in the cat [110]. In the rat however, there is evidence that this pathway does not decussate [57].

**Brainstem nuclei involved in descending inhibition**

In humans, electrical stimulation of the anterior hypothalamus, inferior septal areas and the anterior, mid or posterior periventricular grey sites all produce effective pain control [102]. Stimulation near the arcuate nucleus, while effective, also elevates blood pressure. Stimulation of the centrum medianum nucleus of the thalamus (in the posterior wall of the third ventricle) is commonly used. Stimulus-produced analgesia (SPA) from these sites can be blocked by naloxone, arid thus is mediated at some stage by opioids. Stimulation of the sensory relay nuclei of the thalamus and internal capsule, however, causes naloxone-independent analgesia in humans [102]. It appears that SPA from these higher centres is mediated by activation of brainstem nuclei [26]. Those most strongly implicated are the PAG, the raphe nuclei and the LC.

**THE PERIAQUEDUCTAL GREY**

The story of descending inhibition really starts with the periaqueductal grey (PAG), the first region where electrical stimulation could be shown to evoke a degree of hypoalgesia adequate for surgical intervention [99].

In addition to a topographically organized spinal input [8], the rat PAG receives significant afferents from many nuclei in the diencephalon (thalamic parafascicular nucleus, dorsal premammillary nucleus, zona incerta, hypothalamic dorsomedial and ventromedial nuclei and the ventral part of the anterior hypothalamus, arcuate nucleus) and brainstem (many reticular formation nuclei, NRM, pallidus and obscurus, LC and ventral parabrachial nuclei among others). Weaker inputs come from the medial preoptic area, lateral septum and the anterior cingulate cortex [70, 109, 131].

There are three major projections from the PAG that are central to its role in descending inhibition of pain. First, there is a pathway from PAG to a rostromedial pericoerulear region that includes Barrington’s nucleus but not the LC proper [33]. Second, the PG projects to the nucleus paragigantocellularis (PGi) [120]. Third, and perhaps most important, the PAG sends fibres to the NRM [10].

Many (~ 60 %) NRM raphe-spinal cells in the monkey are excited by PAG stimulation [130]. Electrical stimulation of PAG also inhibits spinothalamic tract (STT) cells in the monkey [38]. The latency of this inhibition is consistent with the possibility that it is mediated by the terminals of the raphe-spinal cells.

Electrical stimulation of the PAG carries the danger of activating fibres of passage (see above). Thus, although stimulation of PAG may suppress the nociceptive responses of dorsal horn neurones [12], it is significant that this can be mimicked in only a proportion (~ 40 %) of horn cells by micro-injection of glutamate into the PAG [17, 55]. There is evidence that different subregions of the PAG may be involved in different forms of analgesia. Opioid analgesia appears to be mediated via the ventrolateral PAG while the lateral portion of nucleus elicits non-opioid analgesia [8].

The pathways mediating PAG inhibition of dorsal horn cells descend mainly in the dorsolateral funiculus (DLF) although there may also be a (species-dependent?) component in the ventrolateral funiculus (VLF) [38]. At least one study has shown that depletion of spinal 5-HT by 5,7-dihydroxytryptamine does not modify analgesia elicited by PAG stimulation [54]. This raises, the distinct possibility that transmitters other than 5-HT (e.g. NA) may be involved or that some of the effects are mediated via direct spinal projections [69]. Interestingly, it has been shown that the effect of PAG stimulation on rat superficial dorsal horn cells is mimicked most closely by NA but not by 5-HT [78].

It is unclear at present whether descending inhibition from the PAG is tonically activated. For instance, one study found that tetracaine block of the PAG was hyperalgesic in the tail-flick test [105] while another failed to show an effect after injection of lignocaine into the PAG [19]. These differences may be resolved with more careful behavioural controls.

**RAPHE NUCLEI**

There are several subdivisions of the raphe nuclei. The two most widely implicated in descending control are the nucleus raphe magnus (NRM) and the dorsal raphe nucleus (DRN). The NRM receives an input from the PAG and it is thought to mediate at least some of the effects of PAG stimulation. Direct stimulation of NRM causes analgesia in behavioural algesiometric tests [84].

Electrical stimulation of the NRM decreases responses of dorsal horn cells to noxious heat [67] while microinjection of glutamate in the NRM exerts the same effect in the majority (~ 70 %) of cases [55]. This has been taken to indicate that the effect of electrical stimulation on the NRM is largely due to cell bodies sited within the NRM. The spinothalamic pathway from the NRM descends mainly via the ipsilateral DLF in the rat [57] and the contralateral DLF in the cat [110].

Despite the high levels of 5-HT in the raphe nuclei, raphe-spinal actions may not wholly be mediated via 5-HT release. A recent retrograde immunohistochemical study showed that only...
around 50% of the serotoninergic neurones in the raphe nuclei and surrounding area project to the lumbar spinal cord and furthermore, that half of the raphe cells that do project are not serotoninergic [58]. There is also a significant amount of NA in the NRM. It is possible that this is a modulatory site since approximately 10% of NRM serotoninergic cells express alpha2 receptors [41]. Interestingly, the NA does not appear to come from coerulear afferents to the NRM and descending inhibition from the LC does not appear to be delayed via the NRM [57]. Evidence for a role of NA in raphe-spinal actions comes from the observation that inhibition of the tail-flick reflex by NRM stimulation can be blocked by intrathecal phenolamine as well as methysergide [43].

The DRN is situated close to but is functionally distinct from the PAG. Electrical stimulation of the DRN (as opposed to the PAG) causes behavioural analgesia [82]. Indeed, it has been suggested that the DRN may be the most effective nucleus for eliciting stimulation analgesia in the cat brain [83]. Both clonidine and morphine injected into DRN have been shown to be analgesic [126].

**NUCLEUS PARAGIGANTOCELLULARIS**

The connections of the nucleus (reticularis) paragigantocellularis (PGi) in the ventromedial medulla are such that it is in a position to play a pivotal role in descending inhibition: the PGi gives rise to a massive projection to the LC [6]. This projection seems to be mediated through both an excitatory amino-acid. pathway and an adrenergic component [32]. The PGi projects to the spinal cord and to the NRM [10, 11]. It also receives a projection from the PAG [120]. Injection of tetracaine into the PAG blocks the effect of microinjected metenkephalin in the PGi [105].

**LOCUS COERULEUS/SUBCOERULEUS**

The rat LC is usually considered the main noradrenergic nucleus involved in the descending control of pain. Its main inputs are from the nucleus prepositus hypoglossi (PrH) and the PGi [6]. It does not receive a direct input from the PAG which instead innervates the pericoerulear region [33] but may influence the LC via the PGi.

It is generally accepted that the LC sends a major projection to the spinal cord in the rat [64] although the precise details of the pathway are unclear. For instance, the LG innervates the ventral horn in Casco Sprague-Dawley (SD) rats while the A7 cell group projects to the dorsal horn [21]. In Harlan SD rats the situation is reversed [23, 35]. A5 goes to the intermediate zone and ventral horn in Harlan SD [22]. Irrespective of final location, the coeruleospinal projection seems to be carried via the VLF. This trajectory is also observed in primates [16].

Noxious stimuli (tail pinch and formalin injection into the foot) have been shown to increase activity of LC cells, as evidenced by a rise in NA metabolites in the LC [48]. Stimulation of the LC/SC causes antinociception, as measured by the hot-plate test [71] and inhibition of heat-evoked dorsal horn activity [56]. Since the effect is also mimicked by intracoerulear application of glutamate, it is not due to activation of fibres of passage [56]. LC stimulation also causes an elevation of spinal cord NA metabolites [25] strongly suggesting the involvement of NA in the antinociceptive effect (see below). Bilateral transection of the DLF has no effect on LC-induced inhibition of dorsal horn cells activated by noxious heat [57], Uni- and bilateral injection of lignocaine in the VLF, however, reduces the response. The projection is mainly but not exclusively ipsilateral [57]. The absence of effect of LC lesions on baseline nociceptive thresholds after electrolytic lesion of the LC [86] suggests that the coeruleospinal pathway is not tonically activated.

Activity of LC cells is under the control of an alpha2 autoreceptor. For instance, dexmedetomidine decreases LC NA efflux and cell firing [60]. Both effects can be antagonized by atipamezole. Furthermore, clonidine suppresses the activity of LC neurones and reduces the morphine withdrawal response [1]. Since both alpha2 agonists and opioids are analgesic, it is clear that their antinociceptive effects are unlikely to be mediated in the LC, since inhibition of cell firing would be expected to have a hyperalgesic action.

Despite the fact that nearly all cells in the LC stain for dopamine tyrosine hydroxylase [116], most LC cells also show GABA-like immunoreactivity and it has been suggested that the LC (at least in the rat) represents a homogeneous population of cells that use NA, GABA and 5-HT as transmitters [51]. In the cat, a major proportion of LC and KF neurones immunostain for glutamate and tyrosine hydroxylase (TH) or enkephalin and TH [35]. Thus, it cannot be assumed that the spinal effects of LC stimulation are wholly mediated by the release of NA in the cord. For instance, LC stimulation causes a decrease in membrane resistance in spinal motor neurones, while NA applied directly increases their membrane resistance [36].

**Transmitters and receptors**

What are the main transmitters involved in descending inhibition and which receptors mediate their actions? In the sense that descending inhibition is multiply relayed, there are many potential sites at which transmitters might act and thus a case can be made for the involvement of a large number of compounds. However, the transmitters most clearly implicated are fewer: NA, 5-HT and the endogenous opioids are the three main candidates.

**NORADRENALINE**

There is a strong body of evidence not only implicating NA in opioid- and central stimulation-induced analgesia but also suggesting that these effects are mediated via descending pathways. It should be emphasized that there are no known NA cell bodies in the spinal cord. In the dorsal horn, NA terminals are mainly concentrated in the upper two laminae in the rat [27]. 6-Hydroxydopamine lesions of the cord dramatically lower NA content and cause hyperalgesia [108] suggesting a functional relation.
Intrathecal NA causes analgesia in the rat [96] and this effect has been shown to be blocked by phenolamine [95]. Intrathecal yohimbine blocks the antinociceptive (tail-flick and hot-plate) effects of intracerebroventricular (i.c.v.) morphine [115] while phenolamine has been shown to block stimulation-induced antinociception [43]. However, phenolamine is a non-selective alpha-adrenoceptor antagonist. Using selective drugs, it has been shown that yohimbine is approximately an order of magnitude more potent as an antagonist of intrathecal NA than prazosin. This implies that alpha_2 receptors are the subtype responsible for NA analgesic effects [107]. Further studies with subtype-specific antagonists indicate that, in rodents, the alpha_{2D} subtype mediates antinociception [76]. The possibility has nevertheless been raised that exogenous NA may act via both alpha_{1A} and alpha_{2A} receptors [76].

Although alpha_{2A} receptors exist both pre- and postsynaptically, the spinal alpha_{1A} sites responsible for antinociception are postsynaptically located. For instance, LC lesions, which halve cord NA content, enhance rather than attenuate the antinociceptive effect of clonidine [86]. Furthermore, intrathecal desipramine potentiates morphine analgesia [65]. Since desipramine elevates extracellular NA concentration, this effect is the opposite of that expected if stimulation of presynaptic alpha_{2A} receptors were responsible for antinociception. There is some evidence that NA uptake block contributes clinically to the effect of some analgesics. For instance, tramadol, the atypical opioid analgesic is a relatively weak opioid agonist yet causes inhibition of spinal NA uptake [98]. At least some of its antinociception may be blocked by yohimbine [92]. Significantly, antidepressants such as amitryptiline and fluoxetine which enhance monoamine transmission, form a cornerstone of current therapy for chronic pain [63].

At the cellular level, the consensus has generally been that NA is inhibitory upon nociceptive transmission and that a spinal alpha_{2A} receptor mediates inhibition of spinal nociceptive cells with ascending axons [34]. However, the synaptology of this effect is relatively complex and may, in part at least, be indirect. For instance, there is evidence that monoamine transporter blockers contribute to the action of NA, it is far from clear which 5-HT agonists act synergistically in the generation of antinociception [28].

**SEROTONIN (5-HYDROXYTRYPTAMINE)**

The involvement of serotonin (5-HT) in descending control of pain has long been recognized: p-chlorophenylalanine (pCPA), which blocks 5-HT synthesis, abolishes central stimulation-induced analgesia, as do lesions of the raphe nuclei made electrolytically or by the selective serotonin neurotoxin 5,6-dihydroxytryptamine (see Basbaum [9]).

Electrical stimulation of NRM has been shown to increase the release/metabolism of 5-HT in the medullary dorsal horn of the rat spinal cord [103] an effect abolished by pCPA pretreatment. Conversely, noxious stimuli (formalin injection into the forepaw) increase 5-HIAA (5-hydroxyindoleacetic acid, a metabolite of 5-HT) concentrations in both the NRM and medullary dorsal horn although these effects are temporally dissociated: the rise in 5-HIAA in the NRM precedes that in the dorsal horn [90]. Systemic morphine increases 5-HT synthesis in the spinal cord, mainly in the dorsal part [104] and antidepressants have been shown to increase 5-HIAA concentrations and potentiate the action of morphine [91].

At the single cell level, 5-HT is generally thought to be an inhibitory transmitter in the dorsal horn. For instance, stimulation of NRM or ionophoresis of 5-HT generally decreases responses to noxious stimuli [44,132]. However, there is some evidence that 5-HT can be excitatory upon small cells (possibly interneurones) in laminae I–III [119]. This is not a non-specific effect since it can be blocked by methysergide [118].

In contrast with the reasonably clear evidence that alpha_{1A} adrenoreceptors mediate the antinociceptive actions of NA, it is far from clear which 5-HT receptors mediate its spinal antinociceptive effects. 5-HT release is at least partly under 5-HT_{1B} control since RU 24969 decreases 5-HIAA concentrations in the medullary dorsal horn [89]. 5-HT_{1A} receptors are implicated in the inhibitory actions of 5-HT as 50–60% of the wide dynamic range cells in the dorsal horn inhibited by PAC stimulation are also inhibited by ionophoresis of the 5-HT_{1A} agonist 8-OH DPAT (8-hydroxy-2-(di-n-propylamino)tetrahydrofuran) and buspirone [332]. However, it is possible (see below) that some of this effect is indirect, mediated via NA terminals.

At the behavioural level, the data are confusing. One study has found that intrathecal 5-HT_{1A} agonists facilitate the tail-flick reflex (i.e. induce behaviour typical of hyperalgesia) while TFMP and CGS 12066B (7-trifluoromethyl-4-(4-methylpiprazinyl)-pyrrolo[1,2-a] quinoxaline) (5-HT_{1B} agonists) prolong the tail-flick latency (i.e. induce apparent analgesia) [3]. Another study found that intrathecal 5-HT_{1A} agonists were antinociceptive whereas 5-HT_{1B} agonists were inactive [80]. Antinociception has also been reported after intrathecal
5-HT₃ agonists [2]. Interestingly, in the light of Millar's suggestion [119] that 5-HT stimulates GABAergic interneurones, GABA antagonists blocked this effect.

Naloxone-induced antinociception, a form of stress-induced analgesia, is also antagonized by 5-HT₃ antagonists (ritanserin and pirenpirone) [122]. Interestingly, activation of spinal 5-HT₂ receptors by intrathecal DOI has been reported by others to lead to an analgesic response (hind-limb abduction) that can be blocked by ketanserin [61].

As well as the projection to the dorsal horn, there is a strong 5-HT projection to the ventral horn, and in this region different receptors may be active. 5-HT depresses the mono- and polysynaptic reflex in neonatal rat spinal cord [123]. 8-OH DPAT preferentially attenuates the monosynaptic reflex [124] although both responses were blocked by ketanserin suggesting that the effects are mediated through 5-HT₂ receptors.

OPIOIDS

Opioids are involved in both ascending and descending components of pain modulation. In the ascending part, all three receptors (mu, delta, kappa) play a part. In the descending component mainly mu and kappa receptors are responsible. Moderate mu-receptor binding is found in the PG, DRN and NRM with higher density in the LC. Mu-receptor mRNA is found mainly in the LC, caudal part of the PAG and DRN. A moderate kappa-receptor density is found in the PAG, DRN and LC while the mRNA is mainly in the DRN and LC [68].

Although systemic morphine is analgesic, there is still debate about the extent to which spinal and supraspinal mechanisms contribute to this effect. For instance, although morphine increases 5-HT release in spinal cord slices, the intrathecal analgesic effect of morphine is unaffected by 5,7-dihydroxytryptamine lesions of spinal 5-HT pathways [121].

Local microinjections of morphine at supraspinal sites have complex and variable actions on dorsal horn cell activity [17]. For example, microinjection into the PAG may inhibit, enhance or not affect responses to noxious stimulation in the dorsal horn [29, 55]. One explanation of this may be the complexity of the organization of the PAG, as has been elegantly demonstrated in the work of Bandler and Shipley [8]. Morphine microinjected into the NRM shows similar paradoxical properties: in some hands, dorsal horn responses to noxious heat are inhibited [55] while others find enhancement [67]. Interestingly, morphine microinjected into the LC/SC inhibits the response of most dorsal horn cells to noxious stimuli [55] despite being known to decrease LC firing [1]. This could reflect a sampling bias in the type of neurones recorded in the LC; or it may partly be a question of dose: for instance, morphine is generally analgesic when given intrathecally but high doses have sometimes been shown to be hyperalgesic [114]. It is possible that this effect may be caused by blockade of alpha₃ receptors [7] as reported in the LC [59].

It is striking that, despite such confusion over its actions in terms of single-unit neuronal responses, morphine injections into PAG or NRM are consistently analgesic in behavioural tests [15, 67]. This heightens the suspicion that sampling bias in neuronal recordings may exist, and, for example, that only physically large cells are detected in many microelectrode recordings. There may also be multiple mechanisms active. For instance, simultaneous injection into PAG and rostroventral medulla (including NRM) of individually subanalgesic doses of morphine is analgesic in combination [106] and blockable by local naloxonazine, the mu antagonist.

Although supraspinal mu-opioid agonists decrease c-fos activation in the dorsal horn and cause analgesia behaviourally [39], there is a danger in assuming that the prototypic opioid agonist morphine behaves in a fashion that can be extrapolated to other opioids. For example, beta-endorphin and morphine appear to have strikingly different synaptology: the analgesic actions (hot-plate and tail-flick tests) of i.c.v. morphine but not beta-endorphin may be blocked by i.c.v. naloxone [115]. Conversely, intrathecal naloxone blocked i.c.v. beta-endorphin but not i.c.v. morphine.

As a footnote, there is evidence that i.c.v. morphine can decrease some components of descending inhibition. Heterotopic application of noxious conditioning stimuli inhibits C-fibre-evoked responses of dorsal horn cells [14]. The fibres that mediate this response descend in the DLF and do not appear to originate in the PAG. Morphine i.c.v. reduces the descending diffuse noxious inhibition of C fibre stimulation in the cord [14]. This mechanism may also operate in humans at therapeutically relevant doses [66].

GAMMA-AMINOBUTYRIC ACID

Approximately 40% of terminals in the PAG are GABAergic and about 50% of PAG cells retrogradely labelled from NRM have GABA-immunoreactive synaptic inputs to their somata and proximal dendrites [97]. Furthermore, about 50% of NRM cells labelled retrogradely from the spinal cord are postsynaptic to GABA-immunoreactive terminals [20] suggesting that rostroventral medulla cells (including NRM) and under GABAergic inhibitory control.

It has been shown that microinjection of the GABAₐ receptor agonist muscimol into either PAG or DRN causes hyperalgesia and also blocks the antinociceptive action of locally applied morphine [81]. Conversely, addition of the GABAₐ antagonist bicuculline in NRM is hypoalgesic [30]. This is consistent with the view that an internal GABA inhibition controls the output of the PAG. The role of GABAₐ receptors is less clear: injection of baclofen into the NRM can be either hypo- or hyperalgesic in the tail-flick test depending on the dose used [42].

ACETYLCOLINE

In contrast with noradrenaline and 5-HT, acetylcholine has been relatively neglected as a candidate
for the mediator of descending inhibitory control. However, nicotine is at least mildly analgesic in animals and humans and it has been shown that microinjection of nicotine into the NRM is antinociceptive in the hot-plate and tail-flick tests [52]. This effect is not blocked by systemic naloxone but is abolished by simultaneous injection of hemicholinium, mecamylamine or pirenzepine. It has been argued that there is a cholinergic projection from the pedunculopontine tegmental nucleus to the NRM [52] that modulates descending inhibition.

Microinjection of carbachol into the DRN is strongly antinociceptive [62]. This is particularly striking as there is little evidence for cholinergic cells or fibres in the DRN [126] which suggests that the effect may not be cholinergic.

Summary

Thanks largely to the study of the brainstem nuclei that mediate stimulation analgesia, the involvement of the monoamines in the descending control of pain is now well established. The periaqueductal grey, the raphe nuclei (NRM and DRN) and the locus coeruleus are all key brainstem sites for the control of nociceptive transmission in the spinal cord. Although the initial emphasis was on 5-HT as the transmitter mediating this control at spinal levels, it is clear from more recent work that NA has an equally important part to play. How (or even if) the two amines differ in their roles and actions in analgesia is, however, still an open question. The small size and complexity of the brainstem areas from which analgesia may be elicited by electrical stimulation complicates the interpretation of the data. Stimulating currents may spread to surrounding regions mediating opposite effects to that of the main region stimulated. Opiates and GABA are clearly involved in descending control at both brainstem and spinal levels, although the relative roles of the different types of amino-acid and opiate receptors is still hotly debated. Despite the fact that the first report on stimulation analgesia appeared more than a quarter of a century ago in 1969, the precise connections and cord synaptology are still the basis of ongoing research. It is perhaps ironic, in an issue dedicated to new molecules and mechanisms, that those transmitters most involved in descending inhibition should be such old and familiar friends.

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Descending control of pain


