Actions of morphine on noradrenaline efflux in the rat locus coeruleus are mediated via both opioid and $\alpha_2$ adrenoceptor mechanisms

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Summary

A recent report showed that morphine inhibited $[^3H]$clonidine binding to human platelet $\alpha_2$ receptors. As the analgesic effects of morphine and clonidine are clinically additive, we investigated the possibility that morphine might stimulate $\alpha_2$ receptors or $\alpha_2$ mechanisms in rat locus coeruleus (LC) slices. Stimulated LC noradrenaline efflux was measured by fast cyclic voltammetry. Cumulatively applied morphine 10$^{-8}$–10$^{-4}$ mol litre$^{-1}$ had no effect on noradrenaline efflux evoked by pseudo-single-pulse stimulations (20 pulses at 100 Hz) while the $\alpha_2$ agonist dexmedetomidine 2 x 10$^{-10}$–10$^{-8}$ mol litre$^{-1}$ decreased efflux of noradrenaline in a concentration-dependent manner. Administration of single concentrations of morphine 10$^{-6}$–10$^{-4}$ mol litre$^{-1}$ significantly decreased efflux of noradrenaline (by 22% and 17%, respectively) and attenuated the effect of dexmedetomidine in a concentration-dependent fashion. Morphine 10$^{-6}$–10$^{-4}$ mol litre$^{-1}$ also decreased efflux of noradrenaline on long stimulus trains (50 pulses at 50 Hz). These data suggest that the analgesic potentiation of $\alpha_2$ and opioid agonists is not mediated via LC $\alpha_2$ receptors. (Br. J. Anaesth. 1995; 74: 73-78)

Key words

The locus coeruleus (LC) is the major brain noradrenergic nucleus with diffuse projections to the entire central nervous system, particularly the forebrain, thalamic nuclei, medulla and the reticular activating system [1, 2], and also descending projections to the dorsal horn of the spinal cord [3]. Coeruleofugal fibres are thought to be important in the regulation of nociceptive transmission. In particular, there is believed to be a dependence on the $\alpha_2$ receptor for opioid-mediated antinociception. For example, the analgesic effects of centrally administered morphine are attenuated by $\alpha_2$ antagonists while conversely intrathecal clonidine (an $\alpha_2$ partial agonist) potentiates the effect of morphine.

The new highly selective $\alpha_2$ adrenoceptor agonist dexmedetomidine is currently undergoing clinical trials as an adjunct to anaesthesia [4]; it may profoundly reduce intraoperative anaesthetic agent requirements [5]. It also reduces requirements for opioid analgesics by up to 80% [6]. Its major site of action is believed to be in the LC [7], although our own work has shown that “non-anaesthetic” $\alpha_2$ adrenoceptor agonists have a similar maximum effect on efflux of noradrenaline in this site [8].

We have been using fast cyclic voltammetry (FCV) to study the effects of dexmedetomidine on noradrenergic neurotransmission in the rat LC and have demonstrated inhibition of both efflux of noradrenaline and LC cell firing rate [9]. In view of the clinical interaction between opioid and $\alpha_2$ receptor function in antinociception, we were interested to investigate if this was manifested at the cellular level in modulation by opioids of the effects of dexmedetomidine at the $\alpha_2$ receptor in the LC.

Materials and methods

BRAIN SLICE PREPARATION

Rats were stunned and then killed by rapid cervical dislocation. No prior anaesthesia was used. The brain was excised rapidly while it was being irrigated with ice cold (−1 to +1 °C) artificial cerebrospinal fluid (ACSF). A vibratome was used to prepare 350-μm thick pontine slices containing the LC (seen as a translucent oval area on the ventrolateral border of the fourth ventricle). The brain slice was secured in a 1-ml tissue bath by a nylon mesh drawn over a stainless steel grid. The internal temperature of the chamber was maintained at 32 °C and the slice was superfused with oxygenated (95% oxygen−5% carbon dioxide) ACSF at 1 ml min$^{-1}$ throughout the experiment [10].

MEASUREMENT OF NORADRENALINE EFFLUX BY FCV

A carbon fibre recording electrode (8 μm diameter) was inserted into the slice, 200 μm from a bipolar tungsten stimulating electrode. Quantitative real-time efflux of noradrenaline was measured using FCV, as described previously [11]. An input voltage of 1.5 cycles of triangular waveform (−1.0 to +1.4 vs Ag/AgCl) at a scan rate of 480 V s$^{-1}$ was applied.

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LABORATORY INVESTIGATIONS

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to the potentiostat every 500 ms. A sample and hold circuit monitored the current at the oxidation potential for noradrenaline (+600 mV).

ELECTRICAL STIMULATION OF NORADRENALINE EFFLUX

For most experiments, efflux of noradrenaline was evoked with trains of 20 pulses (0.2-ms duration and 10 mA constant current) at 100 Hz, applied every 5 min. These short (190 ms) trains minimize autoreceptor activation by released noradrenaline and allow exogenous agonist effects to be seen. The term "pseudo-single-pulse" is often used for these stimulation variables [12]. The ideal would be to use true single-pulse stimulations but, in the LC, noradrenaline efflux is then too small to be measured accurately.

After stimulation, noradrenaline release occurs into the synapse. However, noradrenaline measured by the voltammetric detector electrode in the extracellular fluid is that proportion of the released noradrenaline which has diffused from the synaptic cleft. This is influenced by reuptake and diffusion processes. Hence the term efflux is preferred to the less accurate release.

Some concentration–response curves to morphine and dexmedetomidine were performed with longer trains: 50 pulses at 50 Hz every 10 min. These allow time for autoreceptor activation to occur and thus enable detection of antagonist effects [10]. In other words, noradrenaline released by the first few pulses in the stimulation train is able to act on the autoreceptors to reduce the efflux of noradrenaline produced by subsequent pulses. Therefore, in the presence of an antagonist, net efflux of noradrenaline is increased. Heteroceptor activation also presumably occurs at these stimulation variables as many other transmitters (e.g. GABA, opioids, etc) are probably released in the region of any electrical stimulation.

EXPERIMENTAL PROCEDURES

Three different groups of experiments were performed.

Cumulative morphine and dexmedetomidine concentration–response curves

After a control period of 30 min, incremental concentrations of morphine \(10^{-8}\) or \(10^{-7}-10^{-4}\) mol litre\(^{-1}\) or dexmedetomidine \(2 \times 10^{-10}-10^{-7}\) mol litre\(^{-1}\) were added. Each concentration was left in contact with the slice for 30 min. Electrical stimulation variables consisted of either (a) 20 pulses at 100 Hz applied every 5 min or (b) 50 pulses at 50 Hz applied every 10 min.

Effect of morphine on dexmedetomidine concentration–response relationship

The effect of morphine on the response to dexmedetomidine \(2 \times 10^{-10}-10^{-7}\) mol litre\(^{-1}\) was compared with control responses to the \(\alpha_2\) agonist in the absence of opioid. Stimulation variables consisted of 20 pulse trains (0.2 ms, 100 Hz, 10 mA) applied every 5 min. Morphine \(10^{-8}\) or \(10^{-4}\) mol litre\(^{-1}\) was added to the ACSF 30 min before the first concentration of dexmedetomidine and left in contact throughout the experiment. At the end of each experiment the selective \(\alpha_2\) antagonist atipamezole \(5 \times 10^{-7}\) mol litre\(^{-1}\) was added.

Effect of single morphine concentrations

In some experiments the effect of single (rather than cumulative) concentrations of morphine was examined. Stimulus variables were 20 pulse trains (0.2 ms, 100 Hz, 10 mA) applied every 5 min for six stimulations. Morphine \(10^{-6}\) or \(10^{-4}\) mol litre\(^{-1}\) was added after the first train. In some experiments naloxone or atipamezole (both \(5 \times 10^{-7}\) mol litre\(^{-1}\)) was added 10 min before commencing the stimulation protocol.

DRUGS

Dexmedetomidine and atipamezole were gifts from Farmos (Finland). Naloxone was obtained from Research Biochemicals Incorporated (USA) and morphine sulphate from Sigma Chemical Company Ltd (Dorset, UK).
STATISTICAL ANALYSIS

All noradrenaline efflux data are expressed as percentages of either the pre-drug period (mean noradrenaline efflux on the last three pre-drug stimulations) or, in the case of the single morphine concentration experiments, as the sixth stimulation relative to the first. Data are expressed throughout as mean (SEM) and were analysed by one-way analysis of variance (ANOVA) with post hoc application of Duncan’s new multiple range test unless otherwise stated.

Results

Morphine, added incrementally \((n = 4)\), had no effect on stimulated noradrenaline efflux (Student’s \(t\) test) compared with time-matched controls \((n = 4)\) using the short stimulus train procedure (fig. 1). However, with the long stimulation procedure \((n = 4)\), morphine reduced efflux of noradrenaline compared with drug-free controls \((n = 4)\). This was significant \((P < 0.05)\) at morphine \(10^{-6}\) and \(10^{-4}\) mol litre\(^{-1}\) (unpaired \(t\) test). At morphine \(10^{-6}\) mol litre\(^{-1}\), the difference just failed to reach significance \((P < 0.07)\).

Figure 2 shows the effect of dexmedetomidine \(2 \times 10^{-10}-10^{-7}\) mol litre\(^{-1}\) on efflux of noradrenaline with short and long stimulus trains \((n = 4 each)\). In both cases, dexmedetomidine decreased noradrenaline efflux in a concentration-dependent fashion. However, the \(EC_{50}\) of dexmedetomidine was approximately an order of magnitude lower (i.e. more potent) with the shorter train stimuli. The shorter train was used for all subsequent experiments.

Figure 3 shows the effect of morphine \(10^{-6}\) or \(10^{-4}\) mol litre\(^{-1}\) on the concentration-response relationship for dexmedetomidine. Pretreatment of the slices with morphine, added to the ACSF 30 min before the first dexmedetomidine concentration, caused a concentration-dependent reduction of the response to the \(\alpha_2\) agonist. This was significant at all concentrations of dexmedetomidine except the lowest \((2 \times 10^{-10}\) mol litre\(^{-1}\)). Atipamezole, the selective \(\alpha_2\) receptor antagonist, antagonized the inhibition of noradrenaline efflux by dexmedetomidine to control (pre-agonist) levels, despite the presence (or otherwise) of morphine. Noradrenaline efflux after addition of atipamezole was 119 (19) % in the controls, 99 (5) % with morphine \(10^{-6}\) mol litre\(^{-1}\) and 120 (22) % with morphine \(10^{-4}\) mol litre\(^{-1}\). These values were not significantly different. However, when expressed as a percentage of efflux of noradrenaline after dexmede-
matic up-regulation of adenylate cyclase occurs with efflux by opioid (presumably μ subtype [20]) and action in the rat LC. These include reduction of NA concentration of noradrenaline relative to drug-free controls (fig. 4).

Figure 5 shows the effects of single concentrations of morphine (10^{-6} or 10^{-4} mol litre^{-1}) on efflux of noradrenaline with the short stimulus trains. In the absence of any pretreatment, both concentrations of morphine (n = 9 each) significantly decreased efflux of noradrenaline relative to drug-free controls (n = 77); there was no difference between the two concentrations of morphine. The effects of morphine 10^{-6} mol litre^{-1} were reversed by naloxone 5 x 10^{-7} mol litre^{-1} but not by atipamezole 5 x 10^{-7} mol litre^{-1}. Neither drug prevented the action of morphine 10^{-4} mol litre^{-1}.

Discussion

The locus coeruleus (LC) is a key target for endogenous opioid neurones and there is an extensive body of work examining the effect of opioids on the noradrenergic neurones of the LC. Although there is no evidence of tonic control of LC neurones by opioids [13], exogenously applied μ receptor agonists inhibit LC cell firing via activation of a K channel [14] and inhibition of a slowly depolarizing non-specific cation channel (producing hyperpolarization). Both effects are mediated via G proteins (see [15]).

Opioids also inhibit adenylate cyclase activity and thereby reduce the concentrations of cyclic adenosine monophosphate (cAMP). Even acute morphine administration (2 h) may produce stable inhibition of adenylate cyclase in the LC [16], not requiring the continued presence of the opioid. Conversely, dramatic up-regulation of adenylate cyclase occurs with chronic opioid treatment [17].

The interactions between μ and α2 receptors may be important in addiction [18] and are relevant in the treatment of withdrawal syndromes [19]. In the present study we have shown that morphine has several distinct acute actions on noradrenergic function in the rat LC. These include reduction of NA efflux by opioid (presumably μ subtype [20]) and possibly other receptors, attenuation of α2 agonist action and apparent induction of rapid tolerance.

The data in figures 1 and 5 provide evidence suggestive of acute opioid receptor desensitization or tolerance over a time scale relevant to the acute intrathecal use of opioids: there is a striking difference between the effect of the same concentration of morphine added alone or as part of a cumulative concentration–response curve. When given alone, morphine 10^{-4} mol litre^{-1} significantly decreased efflux of noradrenaline in a naloxone-sensitive manner while, as the third concentration of a concentration–response curve, it had no effect. This suggests that the lower concentrations of morphine are sufficient to desensitize the opioid receptors such that the effect of subsequent larger doses are almost abolished. This explanation may also account for the effect of the higher dose of morphine (the failure of naloxone to antagonize morphine 10^{-4} mol litre^{-1} is possibly caused by the high opioid concentration, 200 times that of naloxone). These effects are presumably mediated via μ receptors as LC cells express a homogeneous population of this opioid receptor subtype [21].

Tolerance or acute desensitization of LC neurones to μ receptor agonists and a reduction in the maximum K+ current produced on re-application have been shown to occur rapidly and to remain for many hours after drug withdrawal [15, 22]. This tolerance may be mediated by a cAMP-dependent pathway [23].

The concentration-related attenuation of the effect of dexmedetomidine suggests, at first sight, that morphine is acting here as an α2 receptor antagonist, a hypothesis consistent with the diminished effect of the recognized α2 antagonist atipamezole (fig. 4). Although unexpected, this possibility is not unprecedented. For example, Limберger, Spath and Starke found that agonists at the κ receptor (the rabbit homologue of the rat presynaptic μ receptor) diminished the ability of clonidine to decrease [3H]noradrenaline efflux in rabbit cortex [24]. Furthermore, in human platelets (which have no endorphin receptors), it has been found [25] that morphine
inhibits the binding of clonidine to \( a_2 \) receptors (1C\textsubscript{50} 4 \times 10^{-4} \text{ mol litre}^{-1})
. Although binding data do not readily distinguish agonists and antagonists, the data are, prima facie, consistent with our own observations.

However, the effect of morphine may also be caused by a post-receptor mechanism. As we have already shown that rapid desensitization of opioid receptor-mediated inhibition of noradrenaline efflux apparently occurs with these morphine concentrations, it is possible that the effect shown in figure 3 represents a form of heterologous desensitization, perhaps mediated in the receptor transduction cascade. For example, it is known that both \( a_2 \) and \( \mu \) receptors are coupled to the inhibitory G protein which opens K\(^+\) channels and that they both can alter cAMP concentrations.

However, in the SH-SY5Y human neuroblastoma line, \( a_2 \) and \( \mu \) receptors are co-localized on the cells and both are negatively coupled to adenylate cyclase [28]. Assays of cAMP demonstrate that the combined effects of morphine and noradrenaline exceed the effect of each agent alone and that no heterologous tolerance (cross tolerance) can be shown after pretreatment with morphine or clonidine. Conversely, Harris and Williams [22] showed modest cross desensitization of LC neurones to noradrenaline after exposure to met-enkephalin for only 5 min. After a longer period of opioid exposure, such as used in this study, the effect may have been greater. Heterologous desensitization may also be specific to different components of the transduction pathway. For example, Christie, Williams and North [27] found no cross tolerance between \( \mu \) receptor and \( a_2 \) receptor agonists on K\(^+\) conductance in LC neurones.

The effect observed with morphine on the long stimulation variables (fig. 1) is intriguing. Here, incremental concentrations clearly did reduce noradrenaline efflux. Two reasons make it unlikely that this is a simple agonist effect at the \( \mu \) receptors on LC cells: first, the long train stimulus design implies that exogenous agonists must compete with higher concentrations of transmitter at their receptor sites than on short trains and this diminishes their effectiveness. This effect is illustrated for dexmedetomidine in figure 2. Second, the fact that a response is demonstrable at all (under circumstances where agonist desensitization has already been shown to occur) makes it unlikely to be mediated via the same receptor.

It is more probable that the diminution of noradrenaline efflux on long trains represents a polysynaptic effect. For example, in addition to causing efflux of noradrenaline, these variables cause stimulation of surrounding neurones and axons and are long enough to allow the released transmitters to also act and possibly alter the efflux of noradrenaline. For example, morphine may act by increasing the release of inhibitory transmitters from interneurones or potentiating their action. Interestingly, we have also observed a similar profile of activity for isoflurane which decreases efflux of noradrenaline on long stimulation trains, but has no action on pseudo-single-pulse trains [28].

Although the locus coeruleus is traditionally considered a cell body region it is worth remembering that is also a major NA terminal region with major inhibitory NA projections to most cells, not least from the contralateral LC. It is possible that there are either different \( a_2 \) receptor reserves or subtypes on the cell bodies and the terminals. It has been found [29] that block of \( a_2 \) receptors increased the firing rate of LC neurones and their responsiveness to a noxious (excitatory) stimulus in a whole animal model. This responsiveness was independent of changes in the spontaneous firing rate and led to the suggestion that two different subtypes of \( a_2 \) receptors may be regulating the LC neurones.

Our own work has also tentatively suggested the existence of \( a_2 \) subtypes or different \( a_2 \) receptor reserves within this nucleus as we found an apparent dissociation of the actions of dexmedetomidine on cell firing and efflux of noradrenaline [9]. It has recently been shown in vitro [30] that the extent of down-regulation of the \( a_2 \) receptor that occurs after exposure to noradrenaline varies among the subtypes. It is possible that the \( a_2 \) receptors controlling efflux of noradrenaline and LC cell firing may differ. This may help explain the disparities in the literature on cross tolerance with opioids.

In summary, we have observed three distinct and, in some ways, counteracting alterations in efflux of noradrenaline after application of morphine to the locus coeruleus slice. First, there was a small, naloxone-sensitive reduction in efflux of noradrenaline [9]. It has recently been shown in vitro [30] that the extent of down-regulation of the \( a_2 \) receptor that is not observed if morphine is applied cumulatively. Second, and at the same concentrations, morphine decreased the action of the \( a_2 \) agonist dexmedetomidine. This is less likely to be caused by competitive \( a_2 \) antagonism than to an allosteric action or disruption of a common transduction mechanism. Third, morphine decreased efflux of noradrenaline on long stimulus trains by a mechanism that is unclear but may be consistent with either stimulation of the complex afferent network present, activation of receptor subtypes, or both.

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References


