ORIGINAL ARTICLE

MODIFICATION OF MORPHINE ANALGESIA BY ALTERING CENTRAL CATECHOLAMINERGIC AND 5-HYDROXYTRYPTAMINERGIC ACTIVITY

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SUMMARY

The roles of central catecholamines and 5-hydroxytryptamine in morphine analgesia were investigated. Drugs that are relatively selective in modifying central monoaminergic activity were used. Nociceptive response was tested by the modified hot plate method. L-Dopa and apomorphine antagonized while chlorpromazine, haloperidol, alpha-methyl-para-tyrosine, and clonidine enhanced morphine analgesia. Para-chlorophenylalanine blocked but L-tryptophan potentiated this effect of morphine. All these changes were significant. The results of the present study indicated that increased brain catecholaminergic or decreased 5-hydroxytryptaminergic activity inhibited the analgesic effect of morphine. On the contrary, decreased central catecholaminergic or increased 5-hydroxytryptaminergic activity enhanced morphine analgesia.

The potent analgesic effect of morphine and other opioids is well-known, however, the mechanism by which they produce this effect is not clear and is still under intense investigation. Since the discovery of opioid receptors (1,2) and later of endogenous opioid peptides (3,4), it is generally accepted that morphine and endogenous opioid peptides bind to the same opioid receptors. Morphine interacts complexly with

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central monoaminergic transmitters and this interaction has been implicated in its analgesic effect (5-10). Although central monoamines can modify morphine analgesia, a consistent pattern has not yet emerged and which monoamine plays a more important role in the analgesic effect of morphine is up for debate.

The present investigation is aimed at verifying the roles of central catecholamines (CA) and 5-hydroxytryptamine (5-HT) in morphine analgesia. Various drugs that alter central levels or activity of these monoaminergic transmitters were used. Analgesic analysis was tested by the modified hot plate method. The results indicated that increased CA or lowered 5-HT activity antagonized morphine analgesia whereas lowered CA or increased 5-HT activity potentiated this effect of morphine.

MATERIALS AND METHODS

Albino rats of either sex weighing between 200 to 300 gm were used. Nociceptive response was measured by placing the animal in a 4 l beaker maintained at 65^{o}C by an automatically temperature controlled water bath. The endpoint of this test was usually taken as licking of hind paws. Some animals, however, displayed either intense agitation or dancing behavior. In these instances, these behaviors were regarded as endpoint. All drugs were administered intraperitoneally and the volume of drug solutions for injection were adjusted approximately 0.3 ml. In the control group, animals were injected with saline, and in the morphine group, animals were injected with 10 mg/kg of morphine sulphate. Test for nociceptive response was done 30 min after saline or morphine treatment.

Other drugs were either freshly prepared or commercially available parenteral forms were used. In the acute experiments, apomorphine (1 mg/kg), chlorpromazine (CPZ, 5 mg/kg), haloperidol (2 mg/kg), and clonidine (50 µg/kg) were injected 10 min after morphine treatment.

In subacute experiments, drugs were administered once daily at 9.00 a.m. for 3 days, morphine treatment was done at 11.00a.m.on the third day. The following drugs were used in subacute experiments: L-dopa (50 mg/kg), alpha-methyl-para-tyrosine (AMPT, 100 mg/kg), para-chlorophenylalanine (PCPA, 100 mg/kg), and L-tryptophan (200 mg/kg). Statistical analysis of the data was done with Student t-test.

RESULTS

Morphine-induced analgesia

In saline-treated group, nociceptive response in the hot plate test was averaged 6.65 sec. Morphine significantly delayed the reaction time to 19.75 sec (Table 1). This represents a three folds decrease in the nociceptive response.

Table 1. Effect of morphine on nociceptive response.

Drugs	Doses (mg/kg)	N	Nociceptive response Mean ± S.E. (sec.)
Saline	-	20	6.65 ± 0.52
Morphine	10	20	19.75 ± 1.02*

* Significantly different from saline treatment, $P < 0.001$

Alterations of central CA activity on morphine-induced analgesia

Effects of drugs that alter CA levels or activity on morphine analgesia were summarized in Table 2. L-dopa significantly antagonized analgesic effect of morphine. Apomorphine, a DA agonist not only completely and significantly blocked morphine analgesia but also seemed

to reverse morphine effect towards hyperalgesia. This seemingly hyperalgesia induced by apomorphine even in the presence of morphine, however, did not reach a significant level when compared with the saline treated-group (Table 1). Markedly and highly significant potentiation of morphine analgesia was observed after a general CA receptor blocker, CPZ. This drug was also tried at higher and lower doses. At higher dose (10 mg/kg) CPZ caused moderate sedation while at lower dose (2 mg/kg), it produced less potentiation on morphine analgesia (data not shown). A more selec-

Table 2. Effects of drugs that alter central CA activity on morphine analgesia.

Drugs	Doses (mg/kg)	N	Nociceptive response Mean ± S.E. (sec.)
Morphine	10	20	19.85 ± 1.42
Morphine + L-Dopa	10 50x3	10	15.70 ± 1.77*
Morphine + Apomorphine	10 1	10	5.80 ± 0.70***
Morphine + CPZ	10 5	10	26.90 ± 2.07**
Morphine + Haloperidol	10 2	10	30.10 ± 1.70***
Morphine + AMPT	10 100x3	10	35.70 ± 1.16***
Morphine + Clonidine	10 0.05	10	25.00 ± 1.11**

* Significantly different from morphine treatment alone, $P < 0.05$
** Significantly different from morphine treatment alone, $P < 0.005$
*** Significantly different from morphine treatment alone, $P < 0.001$

tive DA antagonist, haloperidol showed a greater enhancement of morphine analgesia than CPZ. This enhancement was also highly significant. In addition, AMPT, a depletor of central DA and NE produced the greatest potentiation on morphine analgesia in this study which almost doubled the analgesia produced by morphine alone. Clonidine, a drug that selectively depresses central NE activity was also capable of potentiating morphine analgesia.

Alterations of central 5-HT activity on morphine-induced analgesia

A selective depletor of central 5-HT, PCPA markedly and significantly decreased analgesic effect of morphine. On the contrary, L-tryptophan, a precursor of 5-HT modestly but significantly increased morphine analgesia. The effects of drugs that alter central 5-HT activity on morphine-induced analgesia is shown in Table 3.

All drugs at doses reported in this study by themselves did not produce observable abnormality in general behaviors such as sedation or excitement and did not alter the response to the hot plate test (data not shown).

Table 3. Effects of drugs that alter central 5-HT activity on morphine analgesia.

Drugs	Doses (mg/kg)	N	Nociceptive response Mean ± S.E. (sec.)
Morphine	10	20	19.70 ± 1.55
Morphine + PCPA	10 100x3	10	10.10 ± 1.05**
Morphine + L-tryptophan	10 200x3	10	23.80 ± 1.15*

* Significantly different from morphine treatment alone, $P < 0.025$
** Significantly different from morphine treatment alone, $P < 0.001$

DISCUSSION

Morphine interacts with several transmitters in the central nervous system and this interaction has been implicated in morphine effects including analgesia. There is evidence that morphine can alter central monoaminergic activity (11-13) and that alterations of central monoaminergic function can modify morphine effects (5,6,7,9,10,14). By far, most interest has been focused on the roles of CA and 5-HT in morphine analgesia. Acetylcholine and other non-monoaminergic transmitters such as gamma-aminobutyric acid and substance P may also be important in the analgesic effect of morphine; with the exception of substance P, they receive less attention by most investigators.

In the present study, depletion of central CA levels by AMPT markedly potentiated morphine analgesia, this finding is in agreement with several investigators (6,15). Reduction of CA levels by a neurotoxin, 6-hydroxydopamine (6-OHDA) enhances morphine analgesia (16). Furthermore, blockade of CA receptors by CPZ in the present study potentiated morphine analgesia which is consistent with the work of Takemori et al (9) and Eidelberg and Erspamer (17). On the other hand, increase in central levels of CA by their precursor, L-dopa has been found to antagonize morphine analgesia in this study and others (15,18,19). However, conflicting results have also been generated. Inhibition of morphine analgesia is observed after pretreatment of animals with 6-OHDA (14) while one study found negative effect of AMPT pretreatment (5).

Although the above findings tend to support the role of CA in morphine analgesia, they do not discriminate whether DA or NE is involved in this effect of morphine since those drugs like AMPT, 6-OHDA, L-dopa and CPZ affect both transmitters. There is evidence to indicate that DA plays a more important role in morphine analgesia. Haloperidol, a more selective blocker of DA receptors was very effective in potentiating the analgesic effect of morphine in this study which essentially confirms

the findings of others (9,10,17). On the contrary, apomorphine, a drug that is selective as DA agonist was able to prevent morphine analgesia in this study thereby supporting the observations of Tulunay et al (10), Major and Pleuvry (15), and Vander Wande and Spoerlein (19). In addition, apomorphine not only completely antagonized morphine analgesia but also tended to reverse it towards hyperalgesia. Parenteral administration of higher doses of L-dopa (ED 50 = 115 mg/kg) or apomorphine (ED 50 = 4.4 mg/kg) has been found to produce hyperalgesia in mice (10). At least part of the inhibition of the effect of morphine by these DA agonists is attributable to hyperalgesia induced by these drugs.

There is evidence that NE is also involved in the analgesic effect of morphine. Depletion of central NE levels by DA beta-hydroxylase inhibitors such as 1-phenyl-3-(2-thiazolyl)-2-thiourea (7) or diethyldithiocarbamate (20) is effective in increase morphine antinociception. An alpha-adrenergic blocker, phenoxybenzamine has been found to enhance morphine analgesia (21). This phenoxybenzamine-induced enhancement of morphine analgesia is reversed by naloxone (21). Clonidine activates presynaptic and postsynaptic alpha-2 inhibitory adrenergic receptors thereby reducing central sympathetic activity (22,23). This drug has been found to produce analgesia (24) which exhibits additive analgesic effect to morphine (25). In accordance with the above findings, clonidine was also found to increase morphine analgesia in the present study. Furthermore, bilateral lesions of the dorsal NE bundle potentiate and prolong this effect of morphine (26).

Evidence for the involvement of 5-HT in morphine analgesia appears to be stronger and more consistent than those of CA. Augmentation of 5-HT levels by intraventricular 5-HT (8,18) or by intravenous 5-hydroxytryptophan (27) potentiate morphine analgesia. On the other hand, central depletion of 5-HT by PCPA (5,28) or by 5,6-dihydroxytryptamine (28) blocks morphine analgesia. The results of the present study that PCPA

reduced and L-tryptophan potentiated morphine analgesia further support the above observations. Acute treatment with morphine or heroin enhances central 5-HT synthesis, the latter being more potent than the former (11). Morphine and heroin activate tryptophan hydroxylase, a rate-limiting enzyme thereby leading to increase 5-HT synthesis (11).

Stimulations of many areas of the periaqueductal gray (PAG), periventricular gray, and midbrain raphe nuclei have repeatedly been shown to produce analgesia (29-32) which can be blocked by PCPA (31). Lesions of PAG and raphe nuclei block the analgesic effect of morphine (16,29,33) or produce hyperalgesia (29). There is evidence for an excitatory connection from PAG to midbrain raphe nuclei (34,35), the origin of 5-HT neurons that project to the spinal cord via the dorsolateral funiculus (36,37). Furthermore, PAG contains moderately high levels of opioid receptors and this is the most consistent sites where morphine produces its analgesic effect as reviewed by Feilds and Basbaum (38). These findings strengthen the role of 5-HT in modulation of pain and morphine analgesia.

The results of the present study and many others suggest that reduction of central CA activity, especially that of DA enhance morphine analgesia. On the contrary, increase in DA activity is associated with hyperalgesia and antagonism of the analgesic effect induced by morphine. Furthermore, 5-HT appears to play an important role in pain modulation and in morphine analgesia. Enhancements of central 5-HT transmissions potentiate morphine analgesia while depletions of its levels or lesions of its pathways antagonize this effect of morphine.

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