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GABAERGIC AGENTS MODIFY IMIPRAMINE ANALGESIA

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Abstract : The influence of GABA agonists and antagonists on analgesic activity of imipramine (IMA, 20 mg/kg, ip) was studied using the hot-plate method. Administration of $GABA_A$ receptor agonist muscimol (1 mg/kg, ip), $GABA_B$ receptor agonist baclofen (3 mg/kg, ip) or GABA-T inhibitor aminooxyacetic acid (25 mg/kg, ip) increased the analgesic effect of IMA. On the other hand pretreatment of $GABA_A$ receptor antagonist bicucukline (2 mg/kg ip), $GABA_B$ receptor antagonist δ-amino-n-valeric acid (50 mg/kg, ip) or GABA synthesis inhibitor thiosemicarbazide (50 mg/kg, ip) attenuated the IMA analgesia. These results suggest that the analgesic action of IMA may be mediated by functional alteration of a central GABAergic mechanism and/or subsequent stimulation of GABA receptors.

Key words : imipramine analgesia GABAergic agents

INTRODUCTION

Abundant evidence suggests that tricyclic antidepressants (TCA) have analgesic activity independent of their antidepressant effect (1) and many of these TCAs are used to alleviate chronic pain (2). However, the precise mechanism of analgesic action of TCA is still intriguing. Previous research attempted to correlate TCA-induced analgesia with different neurotransmitter systems like monoaminergic (3) and endogenous opiates (4). However, no information is available about the relation of δ-amino butyric acid (GABA), a major inhibitory transmitter, in the CNS to the analgesic action of TCA. A GABAergic mechanism appears to be involved in antinociceptive processess (5). Many GABA receptor agonists (both $GABA_A$ and $GABA_B$) and antagonists have been shown to have a direct or indirect analgesic potency (6, 7). Moreover, the involvement of GABA in morphine analgesia is well documented (8, 9).

Recently we have demonstrated the influence of calcium channel blockers (10), adenosine (11) and adrenal steroids (12) on imipramine analgesia. In the present study, in order to detect possible interaction between GABA and TCA, the effects of GABAergic drugs on imipramine induced analgesia were studied.

METHODS

Albino mice (Ghosh Co., Calcutta) of either sex weighing 20-25 g were used. They were housed under standard 12h light : dark cycle (lights on at 0700 h) at an ambient temperature of about 23-25°C. Food and water were available *ad libitum*. The pain threshold was assessed (13) using a hot plate analgesiometer (Electrolab, Bomaby, India). The surface temperature of hot plate was maintained at 54±1°C, throughout the experiment. The mouse was placed on the hot plate and either hind paw licking or jumping, whichever appeared first, was taken as the criterian for response. Animals showing reaction time between 10-15 s were used for experiments.

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A cut-off time in the absence of response was 60s in order to avoid tissue injury to the paw.

The drugs used were imipramine hydrochloride (IMA) (Torrent Labs., Ahmedabad), (±) baclofen (BCF) (Research Biochem Inc.), thiosemicarbazide hydrochloride (TSZ) (Burgogyne Burbidges and Co.), muscimol (MUS) (Sigma), (-) bicuculine methiodide (BIC) (Research Biochem Inc.), δ-amino-n-valeric acid (DAV) (Sigma), aminooxyacetic acid hydrochloride (AOAA) (Sigma). All drugs were dissolved in distilled water and were injected intraperitoneally (ip) in a volume of 5 ml/kg. Control animals were treated with an equivalent volume of vehicle.

Mice were injected intraperitoneally with IMA (10-20 mg/kg) and 30 min thereafter the reaction latencies were recorded. In combination studies, MUS (1 mg/kg), BCF (3 mg/kg), BIC (2 mg/kg) or DAV (50 mg/kg) were administered simultaneously with IMA (20 mg/kg). On the other hand, TSZ (50 mg/kg) and AOAA (25 mg/kg) were given 40 min and 60 min prior to IMA respectively. Because maximal effects on GABA synthesis of TSZ (inhibits GABA synthesis) and AOAA (enhances GABA accumulation) occurs at about 70 min (8) and 1.5 h (9) respectively after their administration.

The *per se effects* of GABAergic agents were also studied at an appropriate time.

The data was analysed statistically using Student's 't' test and a probability level of $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Intraperitoneal injection of IMA (20 mg/kg) produced antinociception but at the lower dose (10 mg/kg) IMA had no significant effect. $GABA_A$ receptor agonist MUS (1 mg/kg, ip) and $GABA_B$ receptor agonist BCF (3 mg/kg) produced significant increases in pain threshold as compared to vehicle controls (Table I). On the other hand AOAA, an inhibitor of GABA-transaminase which produces accumulation of GABA in CNS had no analgesic effect in a dose of 25 mg/kg. Similarly, $GABA_A$ receptor antagonist BIC (2 mg/kg, ip), $GABA_B$ receptor antagonist DAV (50 mg/kg, ip) or TSZ (50 mg/kg, ip), an inhibitor of glutamate decaboxylase (which inhibits GABA synthesis) failed to induce antinociception.

TABLE I : Effect of GABAergic agents on imipramine analgesia.

Gps	*Treatment*	*Dose (mg/kg)*	*n*	*Reaction-latency in seconds (Mean ± SEM)*
1.	Control	—	10	10.97 ± 0.99
2.	IMA	10	10	12.59 ± 1.24^{NS}
3.	IMA	20	10	26.08 ± 1.89^{c}
4.	MUS	1	10	20.50 ± 0.96^{c}
5.	MUS+IMA	1+20	8	39.32 ± 1.28^{c}
6.	BCF	3	10	17.73 ± 0.84^{c}
7.	BCF+IMA	3+20	8	35.78 ± 1.19^{b}
8.	AOAA	25	10	9.92 ± 0.55^{NS}
9.	AOAA+IMA	25+20	8	31.47 ± 0.79^{a}
10.	BIC	2	10	9.94 ± 0.53^{NS}
11.	BIC+IMA	2+20	8	17.07 ± 0.98^{b}
12.	DAV	50	10	9.32 ± 0.53^{NS}
13.	DAV+IMA	50+20	8	14.96 ± 0.90^{b}
14.	TSZ	50	10	10.49 ± 0.65^{NS}
15.	TSZ+IMA	50+20	8	18.06 ± 0.74^{b}

n = Number of animals in a group; NS = Not significant; a = $P<0.05$; b=$P<0.01$; c= $P<0.001$ as compared with respective controls. Gps 2, 3, 4, 6, 8, 10, 12, 14 vs 1; Gps 5, 7, 9, 11, 13, 15 vs 3.

However, GABA mimetics, eg. a $GABA_A$ agonist MUS (1 mg/kg) or a $GABA_B$ agonist BCF (3 mg/kg) or GABA-T inhibitor AOAA (50 mg/kg) when given with IMA (20 mg/kg), had a greater analgesic effect than when given alone. On the other hand simultaneous administration of $GABA_A$ antagonists BIC (2 mg/kg) or $GABA_B$ antagonist DAV (50 mg/kg) or prior administration of GAD inhibitor TSZ (50 mg/kg) reduced the analgesic effect of IMA.

The present study replicates the results of several other investigators that many GABAergic agents have intrinsic analgesic potency (6, 7) and also shows that GABA-agonists enhance and GABA-antagonists inhibit the antinocicep-

tive effect of IMA. These observations indicate that the brain GABAergic neurotransmitter system participates in IMA induced analgesia.

Distinct sites of action have been proposed for the analgesic effect of different GABAergic agents, eg. supraspinal site for MUS and spinal and supraspinal site for BCF (14), whereby BCF is reported to act by inhibition of effects of substance P (15). It has also been shown that nociceptive primary afferent endings in the superficial dorsal horn contain very high concentration of $GABA_B$ receptors (16). Some antidepressants (eg. imipramine, amitryptyline, desipramine) have been shown to inhibit GABA uptake *in vitro* (17). However, it has also been observed that acute administration of desipramine and related antidepressants in lower doses do not influence GABA levels and GAD activity (18) but high doses (25-100 mg/kg) markedly increase the GABA concentration in whole mouse brain (19). Perhaps this may explain why the lower dose (10 mg/kg) of IMA was not analgesic. This is further supported by the fact that AOAA (which causes accumulation of GABA) increased while TSZ (which inhibits GABA synthesis) reduced the IMA analgesia.

Thus, functional alteration of GABA system in CNS alone and/or subsequent activation of both GABA receptors may be an important factor for the occurrence of analgesic action of IMA.

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