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α_2-Adrenoceptor- and D_2-dopamine receptor-mediated analgesic response of B-HT 920

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Abstract—The involvement of D_2-dopamine receptors in the antinociceptive action of B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-(4H) thiazolo-(4,5d)-azepine) has been investigated in mice. B-HT 920 (0·1–2·0 mg kg^{-1}) and apomorphine (0·1–2·0 mg kg^{-1}) produced a dose-dependent increase in tail flick latency. Analgesia induced by apomorphine was blocked by the D_2-antagonist, haloperidol (1 mg kg^{-1}) but not by the opioid antagonist, naloxone (1 mg kg^{-1}). The antinociceptive action of B-HT 920 was potentiated by the D_1-agonist SKF 38393 (5 mg kg^{-1}), an action antagonized by haloperidol. The selective α_2-adrenoceptor blocking drug yohimbine (1 mg kg^{-1}) and naloxone (1 mg kg^{-1}) blocked the antinociceptive action of B-HT 920 (1 mg kg^{-1}). Haloperidol, however, failed to modify the B-HT 920-induced increase in tail flick latency. B-HT 920 and apomorphine reversed reserpine (2 mg kg^{-1}) 4 h-induced hyperalgesia. The reversing action of apomorphine was blocked by haloperidol but not by yohimbine. Thus, a role of α_2-adrenoceptors and D_2-dopamine receptors is postulated in the antinociceptive action of B-HT 920.

Considerable evidence suggests that dopamine receptor stimulation in the spinal cord as well as in the supraspinal structures of the central nervous system evokes an analgesic response (Fleetwood-Walker et al 1984; Barasi & Duggal 1985; Fleetwood-Walker & Hope 1985). Non-selective dopamine agonists like apomorphine (Paalzow & Paalzow 1983; Jensen & Yaksh 1984) and the D_2-selective agonist LY 141865 (Barasi et al 1987) have been shown to have antinociceptive activity whilst the D_1-agonist, SKF 38393 (Setler et al 1978) was inactive in naive animals (Ben-sreti et al 1983a). SKF 38393, however, enhanced the nociceptive threshold following supersensitization of the dopaminergic system (Ben-sreti et al 1983b; Barasi et al 1987). An interaction of dopaminergic, noradrenergic and opioid systems has been suggested in modulation of pain perception (Akil & Liebeskind 1975; Pollard et al 1978). Clonidine, B-HT 920 and guanfacine have been shown to evoke an analgesic response in a variety of test procedures presumably by acting as agonists at presynaptic α_2-adrenoceptors (Parale & Kulkarni 1985). Those studies have also indicated the possibility of a link between opioid and noradrenergic systems based on antinociceptive and electroencephalographic data obtained in animals (Parale & Kulkarni 1985; Kulkarni et al 1986).

More recently it has been demonstrated that, in various behavioural paradigms, B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-4H-thiazolo-(4,5d)-azepine), a drug with dopaminergic and adrenergic actions, produces postsynaptic D_2-dopaminergic actions (Chopra & Kulkarni 1988). This action is potentiated by the concurrent administration of SKF 38393 (Pifl & Hornykiewicz 1988). Since we have earlier reported that B-HT 920, like clonidine, induced antinociception that could be antagonized by yohimbine, in the present study we have examined the participation of the D_2 component of B-HT 920 and its modification by SKF 38393 in the analgesic response in naive as well as reserpinized mice. The effects have been compared with a mixed D_1/D_2-receptor agonist, apomorphine.

Materials and methods

Animals. Wistar albino mice (bred in Central Animal House facility of Panjab University), 20–25 g, selected at random, irrespective of sex, were acclimatized to the laboratory conditions with free access to food and water for 24 h before the experiment.

Technique. The nociceptive threshold was determined as the tail flick latencies elicited in response to noxious radiant heat (D'Armour & Smith 1941; Kulkarni 1980). Baseline latencies to tail-flick withdrawal from the radiant heat source (4–6 s) were established. A cut-off time of 12 s was observed to prevent injury to the tail. A minimum of 5 (2 min interval) trials were recorded from each animal before the test. Animals were tested at 5, 10, 15, 30, 60, 90 and 120 min after drug treatment.

Drugs. Drug solutions were made in distilled water, except reserpine which was first dissolved in a few drops of glacial acetic acid and the volume made up with distilled water. All drugs were administered intraperitoneally in a constant volume of 1 mL/100 g of body weight, either alone or in combination. The antagonists were administered 30 min before the agonists. Reserpine (2 mg kg^{-1}) was administered 4 h before the experiment. The selection of doses was based on earlier reports from our laboratory. Each group comprised 5 to 6 animals. The drugs used were: B-HT 920 (Boehringer Ingelheim, Germany) haloperidol (Searle, Skokie, IL, USA), SKF 38393 (1-phenyl-2,3,4,5- tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride; Research Biochemicals Inc., MA), yohimbine (E. Merck, Darmstadt, Germany), reserpine (Loba Chemicals, Bombay, India) and naloxone hydrochloride (Endo, NY, USA).

Statistics. The data were analysed by ANOVA and the differences between the means compared using Student's *t*-test.

Results

Effect of dopamine agonists on reaction to radiant heat in naive mice. Both apomorphine (Table 1) and B-HT 920 (Table 2) displayed significant elevation of tail flick latency in response to radiant heat. However, apomorphine response was not statistically dose-dependent. The peak antinociceptive effect of apomorphine was observed 10 min after drug administration and 30-60 min after the injection of B-HT 920. SKF 38393 failed to alter the tail flick latency in naive mice.

Effect of receptor antagonists on apomorphine- or B-HT 920-induced increase in reaction time to radiant heat in naive mice. The opioid antagonist, naloxone (1 mg kg^{-1}) and the α_2- adrenoceptor blocking drug yohimbine (1 mg kg^{-1}) blocked the increased pain threshold to radiant heat produced by B-HT 920 (1·0 mg kg^{-1}) (Table 2). Naloxone (1 mg kg^{-1}), however, failed to modify the increased tail flick latency produced by apomorphine (0·5 mg kg^{-1}) (Table 1). Haloperidol (1 mg kg^{-1}) blocked the antinociceptive action of apomorphine (Table 1) but not of B-HT 920 (1·0 mg kg^{-1}) in naive mice (Table 2).

Effect of SKF 38393 on increase in reaction time to radiant heat produced by B-HT 920 in naive mice. Concomitant administration of SKF 38393 (5 mg kg^{-1}) and B-HT 920 (0·5 mg kg^{-1})

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Table 1. Antinocicepive action of apomorphine and its modification by naloxone and haloperidol. The response was recorded at peak effect.

Group	Treatment (mg kg^{-1} i.p.)	n	Mean reaction time (s ± s.e.m.) Before	After
1	Apomorphine (0·1)	5	4·26 ± 0·24	5·00 ± 0·33
2	Apomorphine (0·25)	5	4·09 ± 0·17	5·60 ± 0·52[a]
3	Apomorphine (0·5)	5	4·26 ± 0·14	6·30 ± 0·09[c]
4	Apomorphine (1·0)	5	4·16 ± 0·105	6·66 ± 0·22[c]
5	Apomorphine (2·0)	5	4·44 ± 0·16	7·56 ± 0·52[b]
6	Naloxone (1·0) + apomorphine (0·5)	5	4·06 ± 0·20	5·80 ± 0·23
7	Haloperidol (1·0) + apomorphine (0·5)	5	4·20 ± 0·10	5·00 ± 0·5[a]

[a] $P < 0·05$ compared with the control (before) value and between groups 7 and 3; [b] $P < 0·01$ compared with the control (before) values; [c] $P < 0·001$ compared with the respective control (before) values. F-ratio (14,65) = 7·4, $P < 0·05$.

Table 2. Effect of B-HT 920 on reaction time to radiant heat and its modification by haloperidol, naloxone, yohimbine and SKF 38393 in naive mice. The response was recorded at peak effect.

Group	Treatment (mg kg^{-1} i.p.)	n	Mean reaction time (s ± s.e.m.) Before	After
1	B-HT 920 (0·1)	5	5·99 ± 0·29	6·30 ± 0·41
2	B-HT 920 (0·25)	5	5·46 ± 0·22	6·86 ± 0·26[a]
3	B-HT 920 (0·5)	6	6·05 ± 0·23	8·08 ± 0·36[b]
4	B-HT 920 (1·0)	6	6·33 ± 0·45	9·68 ± 0·18[c]
5	B-HT 920 (2·0)	6	5·80 ± 0·12	9·80 ± 0·13[c]
6	Haloperidol (1·0) + B-HT 920 (1·0)	5	5·13 ± 0·12	8·60 ± 0·24
7	Naloxone (1·0) + B-HT 920 (1·0)	6	6·66 ± 0·45	7·80 ± 0·51[a]
8	Yohimbine (1·0) + B-HT 920 (1·0)	5	4·49 ± 0·13	4·80 ± 0·37[b]
9	SKF 38393 (5·0) + B-HT 920 (0·5)	6	5·94 ± 0·30	11·66 ± 0·55[a]
10	Haloperidol (1·0) + B-HT 920 (0·5) + SKF 38393 (5·0)	6	4·69 ± 0·33	6·20 ± 0·54[b]

[a] $P < 0·05$ when compared with the control (before) value and between groups 7 and 4, 9 and 3; [b] $P < 0·01$ when compared with the control (before) value and between groups 8 and 4, 10 and 9; [c] $P < 0·001$ compared with the respective control (before) values. F-ratio (14,65) = 7·4, $P < 0·05$.

produced a significant potentiation of B-HT 920- induced increase in reaction time to radiant heat. The effect of the combination was blocked by haloperidol (1 mg kg^{-1}) (Table 2).

Modification of antinociceptive action of B-HT 920 or apomorphine response by reserpine. Reserpine (2 mg kg^{-1}), administered 4 h before the experiment, produced a significant reduction in pain threshold to radiant heat. B-HT 920 (1·0 mg kg^{-1}), or apomorphine (0·5 mg kg^{-1}), reversed reserpine-induced reduction in tail flick latency. Haloperidol, but not yohimbine (1 mg kg^{-1}), antagonized apomorphine-induced reversal of reserpine-induced hyperalgesia (Table 3).

Discussion

B-HT 920, a non-selective D_2-agonist produced a dose-dependent elevation of tail flick latency in naive mice. Apomorphine, a D_1/D_2-agonist, also displayed dose-depenent antinociceptive activity. The selective D_1-agonist, SKF 38393, however, failed to modify the reaction. This is in agreement with the report of Bensreti et al (1983a) who demonstrated the lack of antinociceptive activity of SKF 38393 in naive mice. Apomorphine has also been reported to possess a biphasic effect (Paalzow & Paalzow 1983). In low doses, it produced hyperalgesia and in high doses, antinociception. Stimulation of the D_2-receptor by LY 141865

Table 3. Effect of haloperidol or yohimbine on antinociceptive action of B-HT 920 or apomorphine in 4 h reserpinized mice. The response was recorded at peak effect.

Group	Treatment (mg kg^{-1} i.p.)	n	Mean reaction time (s ± s.e.m.) Before	After
1	Reserpine (2·0)	5	6·40 ± 0·11	4·10 ± 0·22[b]
2	Reserpine (2·0) + B-HT 920 (1·0)	5	4·10 ± 0·22	6·20 ± 0·18[b]
3	Reserpine (2·0) + apomorphine (0·5)	5	4·16 ± 0·11	5·80 ± 0·30[a]
4	Reserpine (2·0) + haloperidol (1·0) + apomorphine (0·5)	5	3·89 ± 0·31	4·20 ± 0·33[a]
5	Reserpine (2·0) + yohimbine (1·0) + apomorphine (0·5)	5	3·46 ± 0·34	5·40 ± 0·33[NS]

[a] $P < 0·01$ compared with the control (before) value and between groups 1 and 3, 3 and 4. [b] $P < 0·001$ compared between groups 1 and 2. [NS] (not significant) compared between groups 3 and 5. F-ratio (14,65) = 7·4, $P < 0·05$.

has also been shown to produce antinociceptive activity (Barasi et al 1987). The combination of SKF 38393 and B-HT 920 produced a significant increase in the analgesic response due to B-HT 920. The potentiating response of the combination was blocked by the D_2-antagonist, haloperidol. This suggested that the antinociceptive action of B-HT 920 is modulated by the D_1-receptor system although direct D_1-receptor activation (SKF 38393) fails to produce antinociception. Further, the antinociceptive action of B-HT 920 was blocked by the α_2-antagonist, yohimbine and the opioid antagonist, naloxone. Haloperidol blocked the antinociceptive action due to apomorphine, but not that due to B-HT 920. Reserpine, administered 4 h before the experiment produced hyperalgesia that was accompanied by sedation, ptosis and diarrhoea. Reserpine-induced hyperalgesia was reversed by B-HT 920 as well as apomorphine. The reversing action of apomorphine was blocked by haloperidol, but not by yohimbine. These observations indicate that apomorphine-induced antinociception is a D_2-receptor-mediated response. The hyperalgesic action of reserpine could be attributed to its ability to deplete catecholamines or 5-hydroxytryptamine (Kulkarni & Robert 1982) and as such be reversed by dopamine agonists like B-HT 920 and apomorphine. Precursors of catecholamines and 5-hydroxytryptamine have been reported to reverse the behavioural and biochemical changes caused by reserpine (Carlsson et al 1957). The attenuation of the antinociceptive action of B-HT 920 by yohimbine and potentiation by SKF 38393 suggest that B-HT 920-induced antinociception is mediated by α_2-adrenoceptors as well as postsynaptic D_2-receptors. In the dose range employed, B-HT 920 has been reported to produce the classical effects of postsynaptic D_2-receptor stimulation such as stereotypy and locomotion (Chopra & Kulkarni 1988). The post-synaptic D_2-receptor-mediated actions of B-HT 920 are masked by its action on α_2-adrenoceptors in naive animals and become manifested only in the presence of sufficient D_1-receptor stimulation (SKF 38393). The observation that the D_2-antagonist, haloperidol, reduced the response of the combination of B-HT 920 plus SKF 38393 to the same intensity as B-HT 920 itself, which was sensitive to blockade by yohimbine, further supports the above postulate. Thus, the antinociceptive action of B-HT 920 appears to be complex and may involve more than one type of receptor. Furthermore, the failure of haloperidol to block the antinociceptive action of B-HT 920 and the failure of yohimbine to unmask the action of B-HT 920 on postsynaptic D_2-receptors suggests that the antinociceptive action of B-HT 920 could predominantly be an α_2-adrenoceptor-mediated response (Parale & Kulkarni 1985). The blockade of the antinociceptive action of B-HT 920 by naloxone, however, suggests the possibility of the existence of an interaction between α_2-adrenoceptors and the opioid receptor system in the antinociceptive action of drugs.

The present study, therefore, highlights the participation of D_2 and α_2-adrenoceptors in the antinociceptive action of B-HT 920.

References

Akil, H., Liebeskind, J. L. (1975) Monoaminergic mechanisms of stimulation produced analgesia. Brain Res. 94: 279–296

Barasi, S., Duggal, K. N. (1985) The effect of local and systemic application of dopaminergic agents on the tail-flick latency in the rat. Eur. J. Pharmacol. 117: 287–294

Barasi, S., Ben-sreti, M. M., Clatworthy, A. L., Duggal, K. N., Gonzalez, J. P., Robertson, J., Rooney, K. F., Sewell, R. D. E. (1987) Dopamine receptor-mediated spinal antinociception in the normal and haloperidol pretreated rat: effects of sulpiride and SCH 23390. Br. J. Pharmacol. 90: 15–22

Ben-sreti, M. M., Gonzalez, J. P., Sewell, R. D. E. (1983a) Differential effects of SKF 38393 and LY 141865 on nociception and morphine analgesia. Life Sci. 33: 665–668

Ben-sreti, M. M., Gonzalez, J. P., Sewell, R. D. E. (1983b) The influence of selective dopaminergic and cholinergic agonists and antagonists on precipitated opiate abstinence. Alcohol Alcoholism 18: 353–359

Carlsson, A., Lindquvist, M., Magnusson, T. (1957) 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature 180: 1200

Chopra, K., Kulkarni, S. K. (1988) Potentiation of dopamine receptor mediated responses by B-HT 920 in mice. Arch. Int. Pharmacodyn. Ther. 294: 46–55

D'Armour, F. E., Smith, D. L. (1941) A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72: 74–79

Fleetwood-Walker, S. M., Hope, P. J. (1985) A selective antinociceptive effect of the diencephalic dopamine-containing cell group, A11, on dorsal horn neurones in the rat. J. Physiol. 364: 47p

Fleetwood-Walker, S. M., Hope, P. J., Molony, V. (1984) Dopamine inhibits the responses of multireceptive dorsal horn neurones to noxious but not innocuous, cutaneous stimulation in the rat and cat. Ibid. 354: 95p

Jensen, T. S., Yaksh, T. L. (1984) Effects of an intrathecal dopamine agonist apomorphine on the thermal and chemical evoked noxious responses in rats. Brain Res. 296: 285–293

Kulkarni, S. K. (1980) Heat and other physiological stress-induced analgesia: catecholamine mediated and naloxone reversible response. Life Sci. 27: 185–188

Kulkarni, S. K., Robert, R. K. (1982) Reversal by serotoninergic agents of reserpine-induced hyperalgesia in rats. Eur. J. Pharmacol. 83: 325–328

Kulkarni, S. K., Parale, M. P., Nayar, U. (1986) Morphine-like effects of α_2-adrenoceptor agonists on cortical EEG pattern of rats. Ind. J. Exp. Biol. 24: 259–262

Paalzow, G. H. M., Paalzow, L. K. (1983) Opposing effects of apomorphine on pain in rats. Evaluation of the dose-response curve. Eur. J. Pharmacol. 88: 27–35

Parale, M. P., Kulkarni, S. K. (1985) Apparent PA_2 estimates for the antinociceptive receptor. Arch. Int. Pharmacodyn. Ther. 275: 59–64

Pifl, C., Hornykiewicz, O. (1988) Postsynaptic dopamine agonist properties of B-HT 920 as revealed by concomitant D-1 receptor stimulation. Eur. J. Pharmacol. 146: 189–191

Pollard, H., Llorens, C., Schwartz, J. C. (1978) Localization of opiate receptors and enkephalins in the rat striatum in relationship with the nigrostriatal dopaminergic system: lesion studies. Brain Res. 151: 392–398

Setler, P. E., Sarau, H. M., Zircle, C. L., Saunders, H. L. (1978) The central effects of a novel dopamine agonist. Eur. J. Pharmacol. 50: 419–430