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Clonidine – induced behavioural despair in mice: Reversal by antidepressants

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Abstract. The effects of various alpha$_2$ adrenoceptor agonists on forced swimming – induced despair behaviour were studied in mice. Clonidine, B-HT 920 and guanfacine significantly prolonged the total immobility duration. Clonidine-induced behavioural despair was antagonized by prior treatment with yohimbine. The tricyclic antidepressants imipramine, desipramine, trimipramine, amitriptyline, nortriptyline and doxepin, the MAO inhibitor tranylcypromine, and the antimanic agent lithium reversed clonidine-induced behavioural despair. Chronic treatment with imipramine evoked more pronounced reversal as compared to acute treatment. Amphetamine, a psychostimulant, inhibited clonidine-induced enhancement of immobility duration but diazepam, a skeletal muscle relaxant was without any effect. On the other hand, adenosine showed potentiation of the submaximal response of clonidine. These observations suggest that clonidine-induced behavioural despair is probably mediated through its presynaptic action on alpha$_2$ adrenoceptors, resulting in reduced central noradrenergic outflow. The present data proposes a simple test system to induce depression-like syndrome in animals, sensitive to antidepressant therapy.

Key words: Clonidine – Guanfacine – B-HT 920 – Tricyclic antidepressants – Behavioural despair – Mouse

There is considerable evidence to suggest that norepinephrine plays a key role in the pathogenesis of depression (Carlsson et al. 1969; McMillan et al. 1980; Spyraki and Fibiger 1980). The agents which deplete catecholamines or lower noradrenergic turnover in the brain produce depression-like syndrome in animals. Reserpine-induced behavioural depression may be as a result of depletion of both catecholamines and serotonin (Carlsson et al. 1957). Recently, we have shown that in the forced swimming-induced despair test, purine nucleosides prolong immobility duration in mice, probably by decreasing norepinephrine outflow through their action on presynaptic purinoceptors (Kulkarni and Mehta 1985) an effect analogous to clonidine. Clonidine, an alpha$_2$ adrenoceptor agonist, is reported to produce behavioural depression in several test models. It suppresses operant behaviour (Dwoskin and Sparber 1983), avoidance behaviour (Kostowski et al. 1981), produces hypothermia (Kulkarni 1980), antinociception (Parale and Kulkarni 1985) and inhibits exploratory behaviour in animals (Van der Laan et al. 1985). The present investigation was designed to investigate the effect of clonidine on forced swimming-induced despair behaviour and its modification by pretreatment with various antidepressants. The findings were confirmed using two other more specific alpha$_2$ adrenoceptor agonists, viz. guanfacine (Dausse et al. 1983) and B-HT 920 (Mottram 1983).

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Materials and methods

Animals. Male Wistar mice (20–25 g) were obtained from Central Research Institute, Kasauli and acclimatized to the laboratory conditions preceding an experiment. They were supplied with food and water as required. All experiments were performed between 10 a.m. and 4 p.m.

Induction and measurement of immobility. The procedure was essentially the same as described earlier (Kulkarni and Mehta 1985). The animals were forced to swim individually in a glass jar ($25 \times 25 \times 12$ cm^3) containing fresh water of 15 cm height and maintained at room temperature (22–25° C). After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. A mouse was said to be immobile, when it ceased struggling and made minimal movements of its limbs to keep the head above water. The total duration of immobility was recorded during the next 4 min of a total 6 min test. The changes in immobility duration were studied after administering various drugs in separate groups of animals. Each group comprised of a minimum of five animals.

Drugs. The drugs used in the present study were obtained from following drug houses. Clonidine (SG Pharmaceuticals, Baroda), guanfacine (Sandoz Ltd., Basle, Switzerland), B-HT 920 (Boehringer Ingelheim, W. Germany), yohimbine (E. Merck), adenosine (Loba-Chemie Wien-Fischamend), desipramine (Ciba-Geigy), tranylcypromine (SK&F), amphetamine (SK&F), amitriptyline (MSD), nortriptyline (Carter-Wallace, Panjim), reserpine (Loba-Chemie Wien-Fischamend), diazepam (Ranbaxy Laboratories), lithium (E. Merck), imipramine (SG Pharmaceuticals, Baroda), trimipramine (May and Baker), doxepin (Torent laboratories, Ahmedabad), and pentobarbitone (Loba-Chemie, Bombay).

Drug administration. The drugs were either dissolved in distilled water or, if insoluble, dispersed in a suspension of

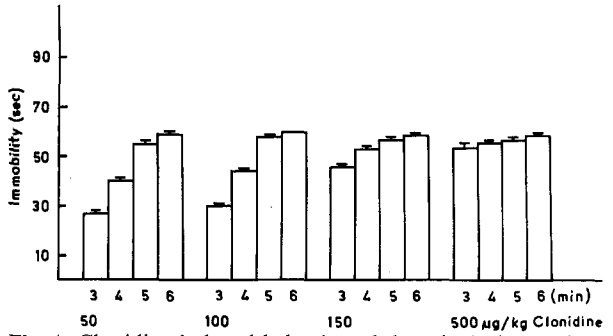

Fig. 1. Clonidine-induced behavioural despair during each minute (3rd min onward) of 6-min test. Each group comprised of a minimum of five animals

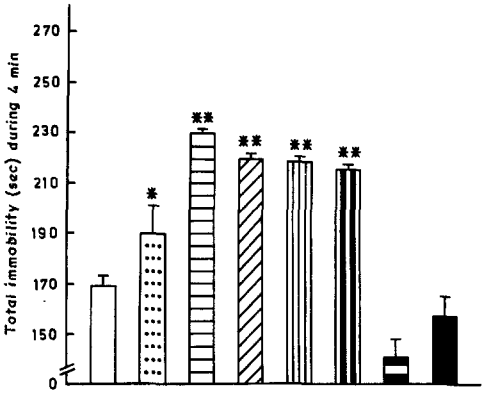

Fig. 2. Histogram illustrates the enhancement in immobility duration induced by adenosine, 25 mg/kg (⊞), reserpine, 2 mg/kg (▤), B-HT 920, 150 µg/kg (▨), guanfacine, 150 µg/kg (▥) and clonidine, 150 µg/kg (▥) in mice as compared to control group (□) and reversal of clonidine action by yohimbine, 0.5 mg/kg (▬) and chronic treatment with imipramine, 10 mg/kg/day for 8 days (■).
$^{*}P<0.01$ and $^{**}P<0.002$ as compared to control group. Each group comprised of a minimum of five animals

carboxymethyl cellulose (0.2% w/v). They were injected IP in a constant volume of 1 ml/100 g. The dose selection of various drugs was based on our earlier studies (Parale and Kulkarni 1985; Kulkarni and Mehta 1985). Imipramine was administered chronically in a dose of 10 mg/kg/day for 8 successive days. Clonidine, B-HT 920, guanfacine and adenosine were administered 15 min before the test whereas, reserpine was administered 2 h, 5 h and 24 h before the test. Rest of the drugs were administered 30 min prior to clonidine treatment.

Statistics. Statistical analysis was done by employing Mann-Whitney two-tailed and Kruskal-Wallis test.

Results

Clonidine significantly ($P<0.002$) prolonged the duration of immobility, in a dose-dependent manner (Figs. 1, 2). Reserpine (2 mg/kg) and adenosine (25 mg/kg) also showed enhancement in total immobility duration (Fig. 2). However, mice treated with reserpine showed ptosis and sedation when tested 2 h and 5 h after treatment. An ineffective dose of adenosine (5 mg/kg) potentiated the submaximal effect of clonidine (50 µg/kg) but did not modify its ceiling response (Table 2). Yohimbine, an alpha$_2$ antagonist reversed clonidine-induced enhancement of immobility duration (Fig. 2). Two other more specific alpha$_2$ adrenoceptor agonists, viz. B-HT 920 and guanfacine, exhibited clonidine-like action on immobility duration (Fig. 2). All the tricyclic antidepressants tested (imipramine, despramine, trimipramine, amitriptyline, nortriptyline and doxepin) caused a reduction in immobility duration in both, control as well as clonidine treated animals (Table 1). Clonidine-induced despair behaviour was also sensitive to reversal by the psychostimulant amphetamine, the antimanic agent lithium and the MAO inhibitor tranylcypromine (Table 1). Chronic treatment with imipramine (10 mg/kg/day) significantly reduced the total immobility duration in clonidine treated mice (Fig. 2). In contrast, diazepam pretreatment had no influence on clonidine-induced helplessness in mice (Table 2). The immobility duration of control animals was not modified when the water temperature was raised from room temperature (25° C) to 40° C. Further, when mice were

Table 1. Effect of various drugs on clonidine-induced behavioural despair during 6-min test (4 min period)

Drug	Dose/kg	*n*	Immobility duration median (s)	*P**
Control	–	10	170	–
Clonidine	150 µg	10	216	$<0.002^{a}$
Imipramine	10 mg	5	139	
Imipramine +Clonidine	10 mg 150 µg	5	190	$<0.01^{b}$
Desipramine	10 mg	5	115	
Desipramine +Clonidine	10 mg 150 µg	5	175	$<0.002^{b}$
Trimipramine	10 mg	5	120	
Trimipramine +Clonidine	10 mg 150 µg	5	150	$<0.002^{b}$
Amitriptyline	10 mg	5	115	
Amitriptyline +Clonidine	10 mg 150 µg	5	166	$<0.002^{b}$
Nortriptyline	10 mg	5	140	
Nortriptyline +Clonidine	10 mg 150 µg	5	173	$<0.002^{b}$
Doxepin	10 mg	5	140	
Doxepin +Clonidine	10 mg 150 µg	5	170	$<0.002^{b}$
Lithium	20 mg	5	123	
Lithium +Clonidine	20 mg 150 µg	10	157.5	$<0.002^{b}$
Amphetamine	1 mg	5	128	
Amphetamine +Clonidine	1 mg 150 µg	5	150	$<0.002^{b}$
Tranylcypromine	10 mg	5	132	
Tranylcypromine +Clonidine	10 mg 150 µg	10	159	$<0.002^{b}$

* As compared to ([a]) control and ([b]) clonidine (150 µg/kg)

NS: Not significant (Mann-Whitney *U*-test)

Table 2. Modification by adenosine and diazepam of clonidine-induced immobility during the 6-min test (4 min period)

Drug	Dose/kg	*n*	Immobility duration median (s)	*P**
Control	–	10	170	–
Clonidine	50 µg	5	181	$<0.025^a$
	150 µg	10	216	$<0.005^a$
	500 µg	5	225	$<0.005^a$
Adenosine	5 mg	5	163	NS[a]
Adenosine +Clonidine	5 mg 50 µg	10	200	$<0.005^c$
Adenosine +Clonidine	5 mg 500 µg	5	224	NS[d]
Diazepam	2 mg	5	170	NS[a]
Diazepam +Clonidine	2 mg 150 µg	5	214	NS[b]

* As compared to ([a]) control, ([b]) clonidine (150 µg/kg), ([c]) clonidine (50 µg/kg) and ([d]) clonidine (500 µg/kg)

NS: Not significant (Kruskal-Wallis test)

treated with a sedative dose of pentobarbitone (30 mg/kg), they were unable to float on water and gradually sank to the bottom of the jar. Unlike reserpine and clonidine, this effect was not modified by imipramine pretreatment.

Discussion

When forced to swim in a confined space, rats or mice after an initial phase of vigorous activity, cease to struggle, surrendering themselves to the experimental conditions. Porsolt et al. (1978) suggested that this helplessness or despair behaviour reflected a state of lowered mood in laboratory animals and could serve as a valuable test for screening antidepressant drugs. We have recently shown that purine nucleosides augmented this helplessness, probably through their presynaptic inhibitory action on noradrenergic nerve terminals (Kulkarni and Mehta 1985). In the present study, clonidine, an alpha$_2$ adrenoceptor agonist, induced a dose-dependent behavioural despair in mice as judged by the significant enhancement in the immobility duration. This characteristic effect of clonidine was reversed by tricyclics, tranylcypramide (MAO inhibitor), lithium (antimanic agent) and amphetamine (psychostimulant). The underlying mechanism for reversal of clonidine-induced behavioural despair by these drugs may be the facilitation of noradrenergic activity resulting from inhibition of norepinephrine uptake, interaction with presynaptic alpha adrenoceptors or MAO inhibition. The reversal of clonidine-induced enhanced immobility could be easily observed and may be made use of to study the antidepressant potential of newer agents. The test procedure is simple enough to lend itself to minor experimental manipulations. The sensitivity of the proposed test system was not modified by the variation in water temperature from 22° to 40° C. Moreover, diazepam, a skeletal muscle relaxant, did not interfere in the test system, thereby supporting its validity.

The sedative as well as the suppressant effect of clonidine on the locomotor activity is likely to overlap the behavioural depressive signs. Recently, we found that the clonidine-induced behavioural depressive signs, observed in the home cage of the rat, were insensitive to acute antidepressant treatment (Parale et al. 1986). The present study indicates that the despair behaviour evoked by clonidine may be dissociable from its sedative effect and is sensitive to antidepressant treatment. It may be worthwhile to note that the animals treated with a sedative dose of pentobarbitone showed a sinking tendency when forced to swim in water, unlike clonidine.

Biochemical and neurophysiological studies indicate that the depressive state produced by clonidine is associated with reduced noradrenergic activity. Tang et al. (1979) and Warsh et al. (1981) found decreased brain 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations in rats treated with clonidine, suggesting reduced turnover of norepinephrine. Furthermore, the rate of firing of ascending noradrenergic neurons in the brain was reduced by both IV injection and direct application of clonidine in the vicinity of the locus coeruleus pericarya (Svensson et al. 1975). In the present study, an ineffective concentration of adenosine potentiated the submaximal effect of clonidine but failed to modify its peak response indicating that the two agents are probably acting at the presynaptic site, regulating norepinephrine outflow. Since yohimbine, which preferentially blocks presynaptic alpha adrenoceptors (Starke et al. 1975) antagonised this action of clonidine, an important role for presynaptic alpha adrenoceptors in the pathogenesis of depression is speculated. Furthermore, guanfacine (Dausse et al. 1983) and B-HT 920 (Mottram 1983), the more specific alpha$_2$ adrenoceptor agonists, also exerted clonidine-like effects.

The present findings propose a simple, sensitive and reliable model to induce depression-like syndrome in animals and underlines noradrenergic involvement in the pathogenesis of depression.

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