Pharmacology Biochemistry & Behavior Vol 24, pp 177–181, 1986 © Ankho International Inc Printed in the U S A 0091-3057/86 $3 00 + 00

Shuttle-Box Deficits Induced by Inescapable Shocks in Rats: Reversal by the Beta-Adrenoreceptor Stimulants Clenbuterol and Salbutamol

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Received 25 March 1985

MARTIN, P , P SOUBRIE AND P SIMON *Shuttle-box deficits induced by inescapable shocks in rats Reversal by the beta-adrenoreceptor stimulants clenbuterol and salbutamol* PHARMACOL BIOCHEM BEHAV **24**(2) 177–181, 1986 — Beta-adrenoreceptor stimulants such as salbutamol and clenbuterol have been reported to be effective in depressive states and to share many actions with classical antidepressants in animals To further explore the antidepressant activity of these drugs, we investigated their effects in rats subjected to helplessness training Rats were first exposed to inescapable shock pre-treatment (60 shocks, 15 sec duration, 1 mA every minute ±15 sec) and 48 hr later, shuttle-box training (30 trials/day, ITI 30 sec) was initiated in order to evaluate escape and avoidance deficits Rats pretreated with inescapable shocks exhibited escape and avoidance deficits when tested for subsequent responding in a shuttle-box The deficits are particularly marked at the third training session Daily IP injections of clenbuterol (total daily dose 0 5 and 0 75 mg/kg) and salbutamol (16 and 24 mg/kg) prevented escape deficits as did daily injections of classical antidepressants such as desipramine (16 and 24 mg/kg/day) and clomipramine (16 and 24 mg/kg/day) These data extend previous results bearing on the similarity of action of beta receptor stimulants and tricyclic antidepressants and further support the notion of a close relationship between noradrenergic function, more especially beta-adrenoreceptors, and "helpless" behavior

Learned-helplessness Escape deficit Antidepressants Beta-adrenoreceptors Clenbuterol Salbutamol Shuttle-box Rats

LEARNED helplessness is a condition in which exposure to an uncontrolled aversive stimulus leads to a decreased ability to escape future aversive situations (see review [12]) For instance, training rats in a grid cage with inescapable electric footshocks results in a subgroup of animals who do not learn to escape subsequent exposure to shocks This model, which is highly sensitive to antidepressant drugs [7, 16, 17, 19, 22], is increasingly used for investigating the mechanisms of action of these agents and the neurobiology of depressive illness [16, 17, 18] In particular, training rats for learned helplessness was shown to increase the density of central beta-adrenoreceptors [6], a change which is reversed by drugs (e g , imipramine) which reduce the escape deficit and cause themselves down-regulation of the number of beta-adrenergic binding sites (see reviews [14, 20, 21]) On the other hand, direct beta-receptor stimulants such as salbutamol and clenbuterol were reported to be effective in depressive states and to share many actions with classical antidepressants in animals [9,10] In the present study the effects of clenbuterol and salbutamol were investigated in rats subjected to helplessness training and compared to classical antidepressant drugs such as clomipramine and desipramine

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METHOD

The experiments were carried out on male Wistar A F rats (Centre d'élevage R Janvier, France) weighing 175–200 g at the beginning of the experiments The animals were housed in groups of 10/cage under standard conditions room temperature (21±1°C), light/dark cycle (12 hr/12 hr), water and food ad lib

Inescapable Shock Pre-Treatment

Electric footshocks were delivered in 25×20×10 cm chambers with Plexiglas walls and cover The floors were stainless steel grids (1 5 cm mesh) A constant-current shocker was used to deliver 60 scrambled, randomized inescapable shocks (15 sec duration, 1 mA, every minute ±15 sec) to the grid flooring Control rats were placed for 1 hour in identical chambers but no shocks were administered Inescapable shock pre-treatment was performed in the morning

Conditioned Avoidance Training

In order to evaluate escape and avoidance performance,

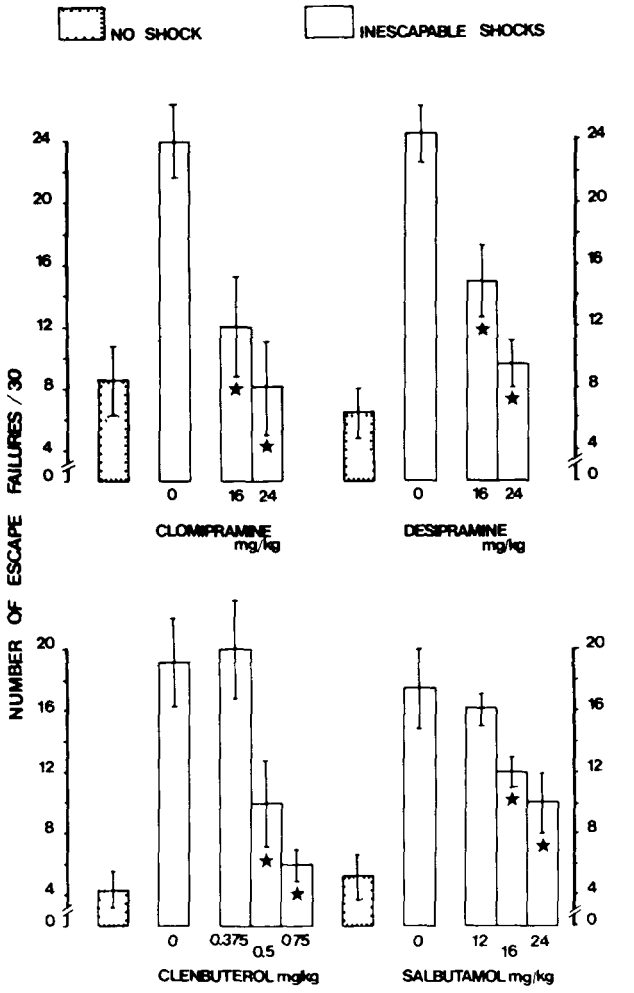

FIG 1 Mean number of escape failures (±SEM) during the 30 trials of the third daily shuttle-box session in control rats and rats pre-exposed to inescapable shock 48 hours before Controls were given saline and rats exposed to inescapable shocks were treated IP with saline, clomipramine, desipramine, clenbuterol or salbutamol during 5 consecutive days Escape failure refers to failure of the rat to change compartments during the electric footshock (1 mA, 3 sec duration) The doses indicated refer to the total daily dose injected on days 2, 3 and 4 (see the Method section) *Indicates that after pre-exposure to inescapable shocks, the response of drug-treated rats differs from that of saline-treated animals ($p<0.05$)

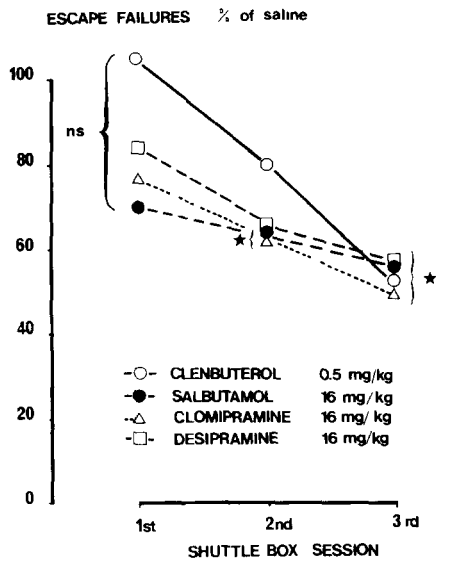

FIG 2 Reversal of escape failures by antidepressant drugs and beta-adrenoreceptor agonists as a function of the number of exposures to daily shuttle-box sessions Escape failures are expressed as a % of the performance of rats subjected to inescapable shocks pre-treatment and injected daily with saline The first shuttle-box session was performed 48 hours after shock pre-treatment The doses indicated refer to the total daily dose administered to the rats on days 2, 3 and 4 (see the Method section) *Indicates that drug-treated rats differ from saline-treated controls at $p<0.05$, n s =not statistically significant The averaged number of escape failures of all control groups was First session 20 0±3 1, second session 21 0±2 4, third session 21 2±2 5

avoidance training was initiated 48 hours after inescapable shock pre-treatment in automated two-way shuttle-boxes (60×21×30 cm) with Plexiglas walls and a floor consisting of stainless-steel rods spaced 1 0 cm apart Each shuttle-box was divided into two equal-sized chambers by a stainless steel partition with a gate providing access to the adjacent compartment through a 7×7 cm space Animals were placed singly in the shuttle-box, allowed to habituate to the test environment for 5 minutes (for the first session only) and then subjected to 30 avoidance trials (inter-trial intervals being 30 sec) During the first 3 sec of each trial, a light signal (used as a CS) was presented, allowing the animals to avoid shock If a response did not occur within this period, a 1 mA shock (3 sec duration) was applied via the grid floor If no escape response occurred within this period, shock and light CS were terminated The response (avoidance or escape) required of the rat was to cross the gate into the other compartment of the box Avoidance sessions were performed for 3 consecutive days in the morning, and the number of avoidances and the number of escape failures were recorded separately for each rat No crossing response during shock delivery was referred to as escape failure, crossing during CS presentation but before shock onset was considered as avoidance

Drug Administration

Rats were randomly treated according to one of the following protocols (16 to 20 rats per group) controls with no shock were given saline, experimental animals with inescapable shocks were injected either with clomipramine, desipramine, clenbuterol, salbutamol or saline The injections were performed during five consecutive days, i e , 6 hr after shock pre-treatment and then, twice a day in the morning (30 min before shuttle-box session) and the afternoon (except the 5th day) Desipramine was given at 16 mg/kg (morning 8 mg/kg + afternoon 8 mg/kg) and 24 mg/kg (8 + 16), clomipramine at 16 (8 + 8) and 24 mg/kg (8 + 16), clenbuterol at 0 375 (0 125 + 0 25), 0 5 (0 25 + 0 25) and 0 75 mg/kg (0 25 + 0 5), salbutamol at 12 (4 + 8), 16 (8 + 8) and 24 mg/kg (8 + 16) The given doses of drugs were chosen within the range usually employed in studies of tricyclic antidepressants [7, 13, 19] or of beta-adrenoreceptor agonists [4,9]

In order to assess possible actions of beta-adrenoreceptor stimulation on shuttle-box performance per se, one group of rats (n=10) with no pre-exposure to shocks was injected

TABLE 1

NUMBER OF AVOIDANCE RESPONSES (% OF CONTROLS ±SEM) DURING 15 MIN AT THE THIRD SHUTTLE-BOX SESSION

	Avoidance responses
Controls (saline)	100 ± 20
Inescapable shock	
saline	26 ± 8
clomipramine	
8 + 8 mg/kg/day	10 ± 5
8 + 16 mg/kg/day	51 ± 15
desipramine	
8 + 8 mg/kg/day	34 ± 10
8 + 16 mg/kg/day	20 ± 6
clenbuterol	
0 25 + 0 25 mg/kg/day	53 ± 25
0 25 + 0 50 mg/kg/day	101 ± 30*
salbutamol	
8 + 8 mg/kg/day	71 ± 19*
8 + 16 mg/kg/day	70 ± 14*

The averaged number of avoidance responses by the rats of all control groups was 8 ± 1 6

*$p<0$ 05 when compared with the associated group subjected to inescapable shocks and given saline

daily with clenbuterol 0 75 mg/kg (0 25 + 0 5) and tested for shuttle-box responding as previously described

Drugs were dissolved in bidistilled water and injected intraperitoneally in a volume of 0 5 ml/100 g b w

Between group comparisons were made with analysis of variance and Dunnett's one-tailed t-test

RESULTS

Analysis of variance revealed that non-drugged rats pre-exposed to inescapable shocks exhibited significantly more escape failures than controls with no shocks, most pronounced differences being observed ($p<0$ 01 for each of the 4 groups) at the third shuttle-box session (Fig 1)

In rats pre-exposed to inescapable shocks, clenbuterol and salbutamol induced a dose-related reduction in the number of escape failures (Fig 1) during the third shuttle-box session, animals treated with clenbuterol 0 5 and 0 75 mg/kg/day and salbutamol 16 and 24 mg/kg/day were significantly different from their saline injected controls ($p<0$ 02, $p<0$ 01, $p<0$ 05, $p<0$ 02 respectively) These changes were similar to those induced at the third shuttle-box session by tricyclic antidepressants such as clomipramine and desipramine Each dose regimen of each drug produced a significant attenuation ($p<0$ 01) of the number of escape failures

Since across-session variations in escape failures by saline-treated shocked animals were minimal, the data presented in Fig 2 indicate that the ability of the 4 compounds to prevent escape failures developed as a function of the repetition of the injections At intermediate doses, all compounds were statistically ineffective in reducing escape failures at the first shuttle-box session and only clomipramine and salbutamol were found to enhance ($p<0$ 02) the ability of rats to escape from shocks during the second shuttle-box session At the highest doses tested, however, all compounds statistically increased the number of escape responses from the first shuttle-box session onwards, although these increases were generally less pronounced than those observed during the last session (data not shown)

Analysis of variance performed on the number of avoidance responses at the third shuttle-box session indicated a significant effect of exposure to shocks and drug treatment (Table 1) In particular, in rats exposed to inescapable shocks, animals treated with beta-receptor agonists but not with tricyclic antidepressants significantly differed ($p<0$ 05) from saline-treated controls in their ability to avoid shock

In addition, none of the drugs were found consistently to affect intertrial shuttling, nor was clenbuterol (0 75 mg/kg/day) able to facilitate shuttle-box responding in rats not pre-exposed to inescapable shocks (not shown)

DISCUSSION

Rats subjected to 60 randomized, inescapable electric footshocks exhibited escape and avoidance deficits when tested for subsequent responding in a shuttle-box Under our experimental conditions, these deficits were particularly marked at the third shuttle-box session, when unshocked control rats were able to avoid a substantial number of shocks or exhibited few escape failures As previously reported, we found that the escape deficit was reduced in rats treated repeatedly with tricyclic antidepressant drugs [7, 16, 17, 19, 22] Likewise, repeated injections of clenbuterol or salbutamol, direct beta-adrenoreceptor agonists with antidepressant activity in humans [10], prevented escape deficits in the animals The deficit was affected rather specifically since stimulation of beta-adrenoreceptors did not cause intertrial shuttling nor facilitate shuttle-box responding in animals that were not subjected to inescapable shock pretreatment The greater activity of clenbuterol compared with salbutamol can probably be accounted for by its reported ability to cross the blood brain barrier more readily [15] These findings which further support biochemical evidence suggesting an intimate—though not exclusive—relationship between noradrenergic function [1,2] mediated through beta-adrenergic receptors [6] and "helpless" behavior deserve several comments

First, two subtypes of beta-receptors, have been identified in the brain, beta 1 and beta 2-receptors and, in light of their central distribution and their sensitivity to various treatments, beta 1-receptors are thought to play a major role in CNS function [14, 20, 21] Our data concerning clenbuterol and salbutamol which stimulate more specifically beta 2-receptors [5,13] did not exclude the possibility of a role of beta 1-receptors in learned-helplessness but extended previous reports suggesting a significant involvement of central beta 2-receptors in the control of animal behavior [4, 9, 13]

Second, blockade of beta-adrenoreceptor with propranolol was not found to facilitate the induction of learned-helplessness [2] The ability of clenbuterol and salbutamol to reverse "helpless" behavior might suggest that the mechanisms by which escape deficits return to normal do not necessarily coincide with the mechanisms by which the deficit was originally produced [17]

Third, drugs which stimulate or inhibit alpha-adrenoreceptors but not drugs acting on beta-receptors have been reported to reduce forced-swimming-induced immobility [8] indicating that, under different experimental conditions, distinct noradrenergic processes may mediate the be-

havioral consequences of exposure to inescapable aversive events

Escape deficit produced by helplessness training has also been associated with reduced activity of central serotonergic neurons [16, 17, 18] Behavioral and biochemical evidence exists to support the possibility that beta-adrenoreceptor stimulants may reverse escape deficits by increasing serotonin function either by enhancing the sensitivity of serotonin receptors to this monoamine or by activating serotonin turnover [3, 5, 23]

Whatever the mechanisms involved in the ability of beta-agonists to reverse learned-helplessness, one of the most interesting findings of this study is that these drugs produced effects similar to those observed with tricyclic antidepressants This verification extends previous results bearing on the similarity of action of beta-adrenoreceptor stimulants and classical antidepressants and may further substantiate the hypothesis of a common, beta-adrenoreceptor—mediated mechanism of "antidepressant" action of these drugs in animals [9, 14, 20, 21] Clomipramine and desipramine however were only able to reduce escape deficit whereas clenbuterol and salbutamol were found to prevent both escape and avoidance deficits in animals trained for helplessness Due to the avoidance procedure used, increases in avoidance responses reduced the number of times rats can be exposed to shock This could be the source of an artifact which could account for the apparent similarity of action between tricyclics and beta-agonists and would fail to support the hypothesis of a common mechanism for these compounds in reversing escape failures This seems not to be the case since the ratio between the number of escape failures/total number of shocks actually delivered at the third shuttle-box session (70% for saline-treated rats) was lowered to similar values by tricyclics and beta-adrenoreceptor agonists (between 30 and 40% for the active doses of the compounds tested)

Taken together our observations are consistent with previous studies indicating that all the behavioral effects of inescapable shock are not equally sensitive to the same drugs [11], and suggest that only the mechanisms involved in the reversal of escape failures are common to both tricyclic antidepressants and beta-adrenoreceptor agonists

Finally, further experiments are needed to ascertain whether repeated administrations of beta-agonists are required to reverse performance deficits induced by inescapable shocks Although this has been proposed for tricyclic antidepressants or MAO Is [17, 19, 22], conflicting data have been reported [7] The present study did not allow us to determine whether repetition of treatment per se or accumulation of critical brain levels of the drugs was the necessary factor in the reversal of performance deficits but our findings clearly indicate that reversal of escape deficits by beta-adrenoreceptor agonists and tricyclics was consistently more pronounced after 8 than after 4 injections, especially for moderate doses of these drugs

ACKNOWLEDGEMENTS

This research was supported in part by a grant from the INSERM (contrat n 133029) Thème "Etats depressifs Ralentissement idéomoteur et systèmes noradrénergiques " PRC Sante mentale et cerveau We thank Boehringer-Ingelheim and Glaxo laboratories for their generous gift of clenbuterol and salbutamol

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