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BRIEF COMMUNICATION

Behavioral Indices of Beta Receptor Subsensitivity After Chronic Treatment With Viloxazine in the Mouse

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PORSOLT, R. D., A. LENÈGRE, S. MIGUEL AND J. LAVOISY. *Behavioral indices of beta receptor subsensitivity after chronic treatment with viloxazine in the mouse.* PHARMACOL BIOCHEM BEHAV **37**(3) 567–570, 1990.—The aim of the experiments was to determine whether chronic pretreatment with viloxazine decreased the sensitivity of mice to the sedative effects of a beta agonist clenbuterol. Mice were subjected to chronic oral treatment with viloxazine (128 mg/kg twice daily) and then given a single administration of 32 mg/kg PO followed by clenbuterol (0.125 mg/kg IP) before being tested in a standard photocell activity meter. Imipramine, administered at the same doses in the same experimental conditions, was used as a comparison compound. The results showed that chronic but not acute viloxazine decreased the hypoactivity induced by clenbuterol, suggesting the induction of beta receptor subsensitivity. With imipramine the results were in the same direction but less clear. The findings are discussed in terms of the eventual specificity of the viloxazine effect to subsensitivity in beta-2 receptors.

Viloxazine Imipramine Chronic treatment Beta receptor subsensitivity Behavioral indices Beta-1 vs. beta-2 specificity

MOST clinically active antidepressants induce an increase in synaptic concentrations of monoamines (dopamine, noradrenaline, serotonin) either by inhibiting their neuronal reuptake or their metabolic breakdown. Although these pharmacological actions have long been thought to be related to the therapeutic mechanism of antidepressants, there exists a poor correlation between them and the clinical time-course; whereas the effects of antidepressants on neurotransmitter transmission are already observed at the first drug administration, the clinical activity of most antidepressants first appears only after several weeks of treatment.

For these reasons numerous researchers have investigated the pharmacological consequences of repeated administration of antidepressants with the aim of discovering a closer link between their pharmacological activity and their therapeutic action. Repeated administration of antidepressants has been shown to induce functional and morphological changes in many neurotransmitter systems (noradrenaline, serotonin, GABA). By far the most consistent change, however, has been the so-called "down-regulation" of beta-adrenergic receptors (17).

Beta "down-regulation" has generally been demonstrated neurochemically either by showing a reduction in the adenyl cyclase response to noradrenaline (18) or by a decreased density of beta-adrenergic binding sites in different brain regions (2). More recently, Przegalinski *et al.* (13,14) have presented behavioral evidence showing that repeated administration of antidepressants to rats attenuated their sedative response to salbutamol, an agonist at beta-adrenergic receptors. This result would appear to provide functional evidence of a decreased responsiveness of beta-adrenergic receptors after repeated antidepressant treatment. Further studies by the same group indicated that similar effects were observed after repeated administration of a nonpharmacological antidepressant treatment, electroconvulsive shock (15). Thus, changes in the behavioral response to beta-adrenergic agonists might represent a simple model for studying adaptive changes in beta-adrenergic function as a consequence of repeated antidepressant treatment.

The present experiments were undertaken to examine the effects of the atypical antidepressant viloxazine in a variant of this model in the mouse. Viloxazine is a derivative of propranolol which, although causing a selective but weak inhibition of noradrenaline uptake (11) and showing antidepressant-like effects in various pharmacological tests (10), differs from classical tricyclics both chemically and by its absence of anticholinergic or cardiotoxic side-effects. Part of its effects may result from direct stimulation of central beta-adrenergic receptors (10).

Mice were repeatedly administered high doses of viloxazine over 11 days and were then challenged with clenbuterol before being tested for spontaneous activity in a standard photocell activity meter. The use of high doses during the pretreatment was aimed to maximise eventual beta down-regulation because the

TABLE 1
SUMMARY OF THE EXPERIMENTAL DESIGN

Description	Pretreatment 11 Days (PO twice daily) 0900 + 1600	Test Treatment on the 12th Day PO −60 min	IP −30 min
Control	Vehicle	Vehicle	Vehicle
Effects of clenbuterol alone	Vehicle	Vehicle	Clenbuterol 0.125 mg/kg^{-1}
Effects of acute viloxazine alone	Vehicle	Viloxazine 32 mg/kg^{-1}	Vehicle
Interaction between acute viloxazine and clenbuterol	Vehicle	Viloxazine 32 mg/kg^{-1}	Clenbuterol 0.125 mg/kg^{-1}
Effects of chronic viloxazine alone	Viloxazine 128 mg/kg^{-1}	Viloxazine 32 mg/kg^{-1}	Vehicle
Interaction between chronic viloxazine and clenbuterol	Viloxazine 128 mg/kg^{-1}	Viloxazine 32 mg/kg^{-1}	Clenbuterol 0.125 mg/kg^{-1}
Effects of acute imipramine alone	Vehicle	Imipramine 32 mg/kg^{-1}	Vehicle
Interaction between acute imipramine and clenbuterol	Vehicle	Imipramine 32 mg/kg^{-1}	Clenbuterol 0.125 mg/kg^{-1}
Effects of chronic imipramine alone	Imipramine 128 mg/kg^{-1}	Imipramine 32 mg/kg^{-1}	Vehicle
Interaction between chronic imipramine and clenbuterol	Imipramine 128 mg/kg^{-1}	Imipramine 32 mg/kg^{-1}	Clenbuterol 0.125 mg/kg^{-1}

phenomenon has been shown to be clearly dose-dependent (16). In contrast to the experiments by Przegalinski *et al.* (13–15), which used salbutamol as beta receptor agonist, the present studies used clenbuterol because this substance is known to penetrate the blood-brain barrier more readily (5). The effects of viloxazine were compared with those of the classical tricyclic antidepressant imipramine, administered in the same experimental conditions.

METHOD

Subjects

Male NMRI mice, 21–31 g, supplied by the Centre d'Elevage R. Janvier, Le Genest St. Isle, France, were used. They were delivered to the laboratory 4 days before the beginning of drug treatment and on arrival were randomly allocated to groups of 12 housed in transparent macrolon cages (25.5 × 19.5 × 13.5 cm) containing purified wood shavings with free access to standard small rodent diet (UAR 113) and tap water.

All experiments were carried out in an ambient temperature of 19–22°C under artificial lighting using a nonreversed light-cycle (lights on between 0800 and 2000).

Drugs

The following drugs were used: viloxazine hydrochloride (I.C.I.); clenbuterol (Boehringer-Ingelheim); imipramine hydrochloride (Cooperation Pharmaceutique Française). Viloxazine and imipramine were dissolved in distilled water (oral administration), and clenbuterol was dissolved in physiological saline (IP administration). All drugs were administered in a volume of 0.25 ml/20 g body weight. Doses are expressed as salt or base as appropriate.

Apparatus

Spontaneous motor activity was measured using a standard photocell activity similar to that described by Boissier and Simon (3). The activity meter consisted of 6 covered Plexiglas enclosures (25.5 × 20.5 × 9 cm) each equipped with two criss-cross photocell assemblies contained within a darkened enclosure and connected to silent electronic counters. The only illumination within the enclosure came from the lights of the photocell emitters.

Procedure

Animals in the chronic pretreatment groups were given two daily oral administrations (0900 and 1600) of either viloxazine (128 mg/kg) or imipramine (128 mg/kg) for 11 consecutive days. The doses indicated were those given at each administration.

On the test day (Day 12), animals received a further oral administration of viloxazine (32 mg/kg) or imipramine (32 mg/kg) followed 30 minutes later by an IP injection of clenbuterol (0.125 mg/kg). All doses were chosen on the basis of pilot experiments to ensure: 1) good tolerance during the pretreatment phase (viloxazine or imipramine: 128 mg/kg PO). 2) A minimum of intrinsic effects of the antidepressants on motor activity during the test (viloxazine or imipramine: 32 mg/kg PO). 3) Clear but not too marked sedation for clenbuterol alone: 0.125 mg/kg IP. Thirty minutes after the injection of clenbuterol, the mice were placed in the activity meters and the number of photocell interruptions was counted during a 10-minute test.

To ensure an unambiguous interpretation of the findings, the experiments contained a certain number of control groups. These, together with the major treatment groups, are indicated in Table 1.

All groups described in Table 1 were run concurrently with all activity meter testing being performed on the same test day. All animals received the same number of administrations throughout the study; when a drug administration was not due the animal received an administration of the appropriate vehicle. The order of testing (Day 12) followed a regular permutation which ensured that the different treatment groups were evenly distributed through-

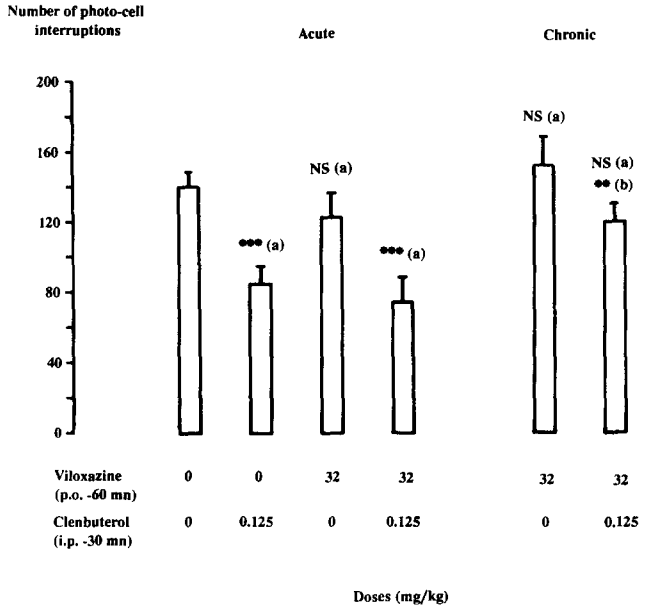

FIG. 1. Effects of acute and chronic viloxazine on the hypoactivity induced by clenbuterol in a photocell activity meter. Chronically treated mice received viloxazine (128 mg/kg PO) twice daily for 11 days and then a single administration of 32 mg/kg on Day 12, one hour before the 10-minute test. Acutely treated animals received the same number of administrations of distilled water. (a) = Compared with the vehicle-treated controls. (b) = Compared with the acute viloxazine + clenbuterol group. Student's *t*-test, NS = not significant; $^{**}p<0.01$; $^{***}p<0.001$ (two-tailed).

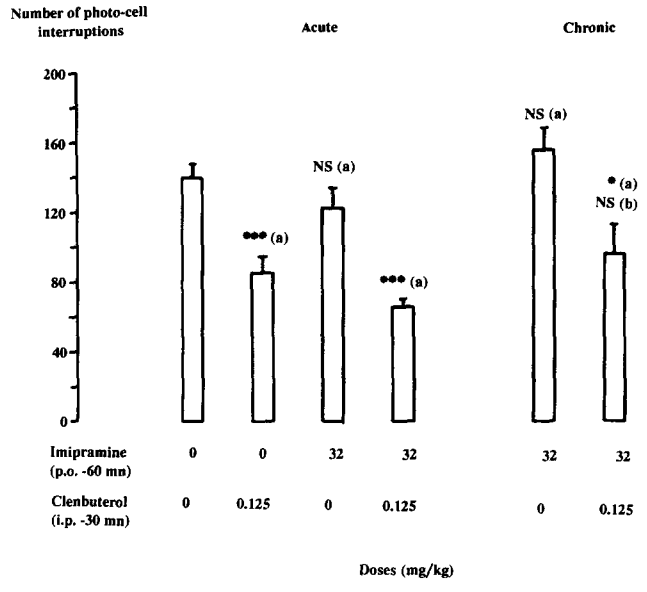

FIG. 2. Effects of acute and chronic imipramine on the hypoactivity induced by clenbuterol in a photocell activity meter. Chronically treated mice received imipramine (128 mg/kg PO) twice daily for 11 days and then a single administration of 32 mg/kg on Day 12, one hour before the 10-minute test. Acutely treated animals received the same number of administrations of distilled water. (a) = Compared with the vehicle-treated controls. (b) = Compared with the acute imipramine + clenbuterol group. Student's *t*-test, NS = not significant; $^{*}p<0.05$; $^{***}p<0.001$ (two-tailed).

out the day. At no time were two animals which had received the same treatment placed in two adjacent cages in the activity meter. Each experimental group contained 12 animals; all animals within a treatment group were housed in the same cage. The results were analyzed statistically using unpaired *t*-tests between appropriate experimental groups.

RESULTS

The results obtained with viloxazine are shown in Fig. 1. Clenbuterol (0.125 mg/kg IP), administered alone 30 minutes before the test, induced a statistically significant decrease in the number of light beams crossed by the mouse during the 10-minute test in the activity meter (hypoactivity induced by clenbuterol).

Viloxazine, administered either acutely (32 mg/kg PO 60 minutes before the test) or chronically (128 mg/kg PO twice daily for 11 days and 32 mg/kg PO 60 minutes before the test) did not affect the number of light beams crossed as compared with control (absence of intrinsic effects of viloxazine). A single injection of viloxazine (32 mg/kg PO 30 minutes before clenbuterol) did not affect the decrease in motor activity observed with clenbuterol alone (absence of attenuation of clenbuterol-induced hypoactivity by acute viloxazine). In contrast, after chronic pretreatment with viloxazine (128 mg/kg PO twice daily for 11 days and 32 mg/kg PO 60 minutes before the test), the decrease in motor activity observed with clenbuterol alone was no longer present (attenuation of clenbuterol-induced hypoactivity after chronic viloxazine).

The results obtained with imipramine are shown in Fig. 2. For purposes of clarity, the data obtained in the neutral control group and in the group treated with clenbuterol alone (see Fig. 1) are reproduced in Fig. 2. Imipramine, administered either acutely (32 mg/kg PO 60 minutes before the test) or chronically (128 mg/kg PO twice daily for 11 days and 32 mg/kg PO 60 minutes before the test) did not significantly affect the number of light beams crossed as compared with control (absence of intrinsic effects of imipramine). A single injection of imipramine (32 mg/kg PO 30 minutes before clenbuterol) did not affect the decrease in motor activity observed with clenbuterol alone (absence of attenuation of clenbuterol-induced hypoactivity by acute imipramine). After chronic pretreatment with imipramine (128 mg/kg PO twice daily for 11 days and 32 mg/kg PO 60 minutes before the test), the decrease in motor activity observed with clenbuterol alone was somewhat reduced but not significantly so; the decrease in activity in this group was still significantly different from the neutral control group (absence of clear attenuation of clenbuterol-induced hypoactivity after chronic imipramine).

DISCUSSION

Taken together, the present experiments provide clear evidence of an attenuation of clenbuterol-induced hypoactivity in mice after chronic treatment with viloxazine. This effect would appear to be specific to chronic treatment with viloxazine because a single injection of viloxazine had no effects on clenbuterol-induced hypoactivity. Furthermore, the doses studied with viloxazine were themselves without intrinsic effects on motor activity in the test situation. The results obtained would therefore be consistent with the notion of a reduced functional activity of the beta-adrenergic receptor after chronic viloxazine treatment.

The results obtained with imipramine in strictly comparable conditions were considerably less clearcut. Although a similar tendency was observed, the decrease in activity induced by clenbuterol alone was still statistically significant even after chronic administration of relatively high doses of imipramine. It

seems unlikely that the absence of effect of imipramine in the present experiments was due to too low doses having been used. The pharmacological literature (4, 10, 12) suggests that imipramine is at least as potent if not considerably more potent than viloxazine. Furthermore, neurochemical investigations of adenylate cyclase activity or the density of beta receptor binding sites suggest that clear indices of beta receptor "down-regulation" can be obtained after chronic administration of considerably lower doses of imipramine than those used in the present experiments (1). Finally, Frances *et al.* (7) have also reported a similar absence of attenuation of clenbuterol's sedative effects in rats after longer term treatment (18 days) of lower doses (10 mg/kg twice daily) of imipramine, an injection schedule similar to that described in the previously cited publication (1).

A possible explanation of the differences between imipramine and viloxazine in the present experiments may arise from differences in receptor specificity between the two compounds. Most studies of beta "down-regulation" have suggested that chronic administration of classical antidepressants and electroconvulsive shock results in a decrease of beta-1 receptor subtypes (6,8). On the other hand, clenbuterol has been shown to have a greater affinity for the beta-2 receptor; indeed, repeated administration of clenbuterol has been demonstrated to induce a specific "down-regulation" of beta-2 sites in cerebral cortex and cerebellum without down-regulating central beta-1 receptors (9). It seems plausible therefore that the failure to observe behavioral signs of beta "down-regulation" in the present experiments with imipramine may have resulted from the specifically beta-2 agonist properties of clenbuterol. If this hypothesis is correct, the present data would suggest a subtle difference in the mechanism of action of viloxazine as compared with more classical antidepressants.

Whatever the explanation, the present findings in mice appear generally consistent with those reported by Przegalinski and his colleagues in rats (13,14) and suggest that the behavioral procedures described could usefully supplement traditional neurochemical techniques for evaluating changes in central beta-adrenergic function.

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