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Reserpine-Induced Hypothermia: Participation of β1 and β2 Adrenergic Receptors

HENRIETTE FRANCÈS AND PIERRE SIMON

Département de Pharmacologie, INSERM U 302, Faculté de Médecine Pitié Salpêtrière 91, Boulevard de l'Hôpital, 75634 Paris Cedex 13, France

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FRANCÈS, H. AND P. SIMON. *Reserpine-induced hypothermia: Participation of β1 and β2 receptors.* PHARMACOL BIOCHEM BEHAV 27(1) 21–24, 1987.—In mice, reserpine-induced hypothermia is partly antagonized by clenbuterol, a beta-adrenergic agonist specific for beta 2 receptors, and completely antagonized by dobutamine, a beta-adrenergic agonist specific for beta 1 receptors. In addition, the effects of dobutamine and of clenbuterol are impaired by betaxolol (1 and 4 mg/kg) and unchanged by ICI 118 551 (1 and 4 mg/kg) beta-blocking drugs respectively selective for beta 1 and beta 2 receptors. These results indicate that reserpine-induced hypothermia depends on the beta 1 receptors and lend support to an indirect effect of clenbuterol. After a chronic treatment, clenbuterol-induced antagonism of reserpine hypothermia is facilitated. This facilitation is impaired by ICI 118 551 and by betaxolol but, in this last case, with high doses only. So the facilitation involves beta 2 adrenergic receptors and implies an increase in the sensitivity of beta 1 receptors.

Reserpine hypothermia Beta 1, beta 2 receptors

CLENBUTEROL is a beta-adrenergic agonist, the specificity of which for beta 2 receptors is particularly high [3]. It crosses easily the blood brain barrier because of its great lipophilicity. Its psychopharmacological profile shows some similarities with that of imipramine [6] and its activity in depressive states has been described by Simon *et al.* [14]. After repeated treatments, clenbuterol's effects are modified: some of them disappear, the others are increased [7]. The explanation for tachyphylaxis is likely a down-regulation of beta-adrenergic receptors. Several hypotheses have been proposed to understand facilitation: one of them is the possible relation between β1 and β2 receptors. These receptors differ in different ways. They are unequally distributed in the brain [11] and do not develop at the same rate [12]. The beta 1 receptor would be the only one innervated [8] and after chronic imipramine treatment only the beta 1 receptors can lead to hyposensitivity.

Reserpine-induced hypothermia is one of the tests that lead to facilitation after chronic clenbuterol. In this work, we tried to specify the relation between beta 1 and beta 2 receptors on reserpine hypothermia regarding two aspects: firstly the antagonism exerted by acute clenbuterol and dobutamine (agonist specific for beta 1 receptors) and secondly the facilitation of clenbuterol's effect after chronic treatment. For this purpose, we used specific drugs for beta 1 receptors: betaxolol [10], and for beta 2 receptors: ICI 118 551 [9].

METHOD

Animals

Male Swiss NMRI mice (20–24 g), from CERJ-Genest St Isle 53940, France, were used in all experiments. Mice were housed in groups of 10 with a 12 hours light-dark schedule in a room thermostatically maintained at 21±1°C. Food and water were freely available.

Reserpine Hypothermia

Acute clenbuterol or dobutamine. Reserpine (2.5 mg·kg^{-1} IP) was administered to mice housed together in their usual cages. Rectal temperature was measured 3 hours after reserpine and mice were selected so as to obtain the same mean rectal temperature in each group. Clenbuterol (0.5 mg·kg^{-1} IP) or dobutamine (16 mg·kg^{-1} IP) were administered 4 hours after reserpine and rectal temperature was measured one hour later. The beta-adrenergic blocking drugs were IP administered 15 minutes before clenbuterol. Controls received water.

Chronic clenbuterol. Mice received clenbuterol (0.25 mg·kg^{-1} IP) twice a day (9 a.m., 6 p.m.) during 5 consecutive days and, after stopping during the weekend, they received the same treatment on days 8 to 11. The last "chronic" treatment was given on day 12 in the morning. Controls received chronic water. The experiment was performed in the afternoon of day 12 with the test-dose of clenbuterol: 0.5 mg·kg^{-1}, according to the protocol described in the acute clenbuterol or dobutamine section, approximately 6 hours after the last chronic administration.

Drugs

The drugs used were clenbuterol hydrochloride (Boëhringer-Ingelheim), dobutamine hydrochloride (Lilly), betaxolol (Synthelabo), ICI 118 551 (I.C.I.) and reserpine

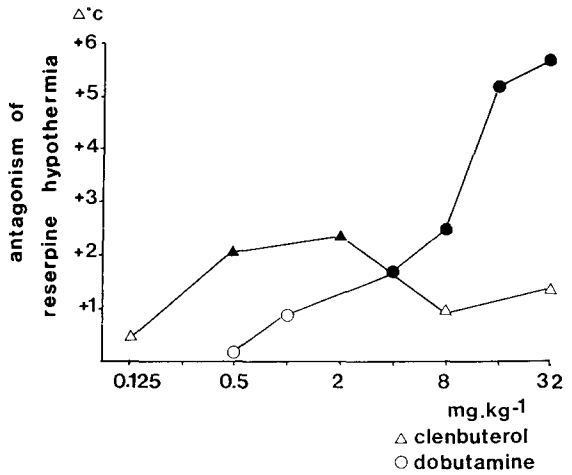

FIG. 1. Effect of clenbuterol and dobutamine on reserpine hypothermia. Reserpine was administered 4 hours before the drug to be studied. Rectal temperature was measured 60 minutes after clenbuterol or dobutamine administration. The values reported are the difference (Δ°C) between the control (reserpine + water) and the treated (reserpine + dobutamine or clenbuterol) groups. The mean rectal temperature (°C±S.E.M.) of control mice was 37.2±0.3 before reserpine treatment and 31.1±0.5 60 min after water injection. Seven to 9 mice in each group. Clenbuterol: $F(5,36)=3.10$, $p<0.05$; Dobutamine: $F(5,36)=30.53$, $p<0.001$. Open symbols: difference non significant. Full symbols: $p<0.01$.

(Sigma). ICI 118 551 was suspended in arabic gum and reserpine, firstly dissolved in acetic acid and kept cool (0°C), was dissolved in water just before use. The other drugs were dissolved in demineralized water.

Statistical Analysis

Data were analyzed using a one way analysis of variance followed by *t*-tests.

RESULTS

Effect of Clenbuterol and Dobutamine on Reserpine Hypothermia (Fig. 1)

Clenbuterol partly antagonizes reserpine hypothermia and the effect disappears with the highest doses 8 and 32 mg·kg^{-1}. The antagonism exerted by dobutamine is dose-dependent and nearly complete.

Effect of the Beta-Blocking Drugs on Reserpine Hypothermia (Table 1)

The reserpine-induced hypothermia was unchanged with the smallest doses (1 and 4 mg·kg^{-1}) but increased with the highest dose (16 mg·kg^{-1}) of the two beta-blocking drugs betaxolol and ICI 118 551.

Antagonism of Acute Dobutamine's Effect

Dobutamine-induced antagonism of reserpine hypothermia was significantly reduced by betaxolol at 4 and 16 mg·kg^{-1} and by ICI 118 551 at 16 mg·kg^{-1} (Fig. 2A).

Antagonism of Acute Clenbuterol's Effect

Clenbuterol-induced antagonism of reserpine hypothermia was completely impaired by betaxolol at 1, 4 and 16 mg·kg^{-1} (Fig. 2B). Clenbuterol-induced antagonism of reserpine hypothermia was unchanged by ICI 118 551 at 1 and 4 mg·kg^{-1} and significantly reduced by the dose of 16 mg·kg^{-1} (Fig. 2B).

TABLE 1

EFFECTS OF THE BETA-BLOCKING DRUGS BETAXOLOL AND ICI 118 551 ON RESERPINE HYPOTHERMIA

	Mean Rectal Temperature °C ± S.E.M.				
	betaxolol mg·kg^{-1}	n		ICI 118 551 mg·kg^{-1}	n
0	31.9 ± 0.5	8	0	31.2 ± 0.5	6
1	30.8 ± 0.4	8 $p>0.05$	1	31.6 ± 0.4	6 $p>0.05$
4	30.8 ± 0.6	8 $p>0.05$	4	31.7 ± 0.4	6 $p>0.05$
16	29.2 ± 0.7	8 $p<0.01$	16	30.0 ± 0.3	9 $p<0.05$

Reserpine and the beta-blocking drugs (or water for the controls) were administered respectively 5 hours and 75 minutes before rectal temperature measurement. The values reported are the mean rectal temperature ± S.E.M. for the numbers of mice reported (n).

For betaxolol, $F(3,28)=3.69$, $p<0.05$; for ICI 118 551, $F(3,23)=5.45$, $p<0.01$.

Chronic Clenbuterol

After chronic treatment with clenbuterol, the effect of a test-dose of the same drug is more important: this is the facilitation. The antagonism is 4.3°C (Fig. 3) and 5.3°C (Fig. 4) in the case of chronic treatment, and 2.5°C or 1.8°C (Fig. 2B) in the case of acute treatment.

This facilitation is not impaired by betaxolol with a dose of 1 mg·kg^{-1} (Fig. 3), a dose which abolished the effect of acute clenbuterol. However, the facilitation is reduced with the higher doses of 8 and 16 mg·kg^{-1}. It is antagonized by ICI 118 551 (Fig. 4). This effect is highly significant with the doses of 1 and 4 mg·kg^{-1} which are inactive against acute clenbuterol.

It is noticeable that beta-blocking drugs increased significantly reserpine hypothermia when animals were chronically treated with clenbuterol but not in acute experiments except with 16 mg·kg^{-1}.

DISCUSSION

Beta-adrenergic agonists antagonize reserpine-induced hypothermia [1, 4, 13]. This effect is a beta-adrenergic one because it is impaired by propranolol [5]. The present results demonstrate that beta 1 receptors are primarily and beta 2 receptors secondarily involved in reserpine hypothermia since the effect of dobutamine (beta 1 selective agonist) is clearly dose-dependent and nearly complete whereas the effect of clenbuterol (beta 2 selective agonist) is significant but small and does not increase with the doses. In addition, the effects of clenbuterol or dobutamine on reserpine hypothermia are antagonized by betaxolol (beta 1 selective antagonist) at doses inactive against reserpine hypothermia. On the contrary, the effects of clenbuterol or dobutamine on reserpine hypothermia are unmodified by ICI 118 551 (beta 2 selective antagonist) except at the dose of 16 mg·kg^{-1} which

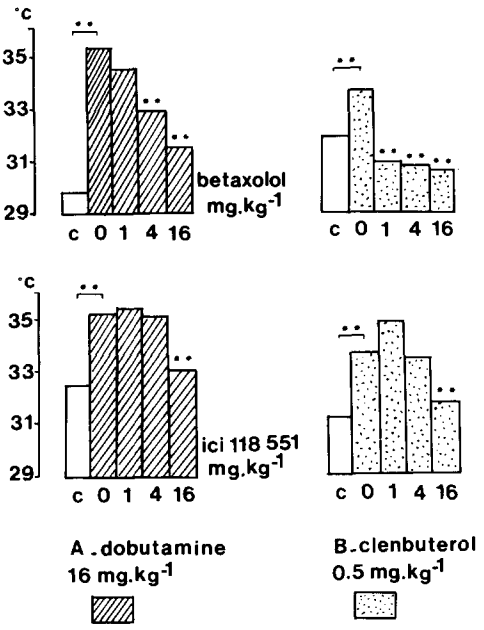

FIG. 2. Interaction of betaxolol and ICI 118 551 with clenbuterol and dobutamine on reserpine hypothermia. Reserpine and the beta-blocking drugs betaxolol or ICI 118 551 were administered respectively 4 hours and 15 minutes before clenbuterol or dobutamine. Rectal temperature was measured 60 minutes after clenbuterol or dobutamine administration. The values reported are the mean rectal temperature±S.E.M. for 6–9 mice in each group. c: clear bars =reserpine + water + water. 0=reserpine + water + dobutamine or clenbuterol. Hatched bars: dobutamine 16 mg·kg^{-1}. Spotted bars: clenbuterol 0.5 mg·kg^{-1}. The asterisks upon the columns relate to the controls 0. $^{**}p<0.01$. Dobutamine + betaxolol: $F(4,36)=29.04$, $p<0.001$; Dobutamine + ICI 118 551: $F(4,25)=7.05$, $p<0.001$; Clenbuterol + betaxolol: $F(4,35)=9.17$, $p<0.001$; Clenbuterol + ICI 118 551: $F(4,28)=15.55$, $p<0.001$.

is probably the result of its proper effect on reserpine hypothermia.

The facilitation of clenbuterol's effect, obtained after chronic treatment, is impaired by ICI 118 551. It is also antagonized by betaxolol but not for the dose of 1 mg·kg^{-1} which is active against acute clenbuterol. Betaxolol becomes active with higher doses (8 and 16 mg·kg^{-1}). So, it appears that facilitation involves beta 2 receptors and in addition an increase in the sensitivity of beta 1 receptors.

These results are surprising since they show that clenbuterol, generally regarded as an agonist highly specific for beta 2 receptors [2,3] seems to act also on beta 1 receptors. This action may be indirect: clenbuterol, by stimulating presynaptic beta 2 receptors, may induce a release of noradrenaline which would stimulate post-synaptic beta 1 receptors. According to this hypothesis, a chronic treatment would lead to a down-regulation of presynaptic beta 2 receptors which becoming less reactive to physiologically released noradrenaline would reduce their function of noradrenaline releasers, inducing in turn hypersensitivity of post-synaptic beta 1 receptors.

The presynaptic beta 2 receptors although desensitized may not be unresponsive to the stimulant effect of clenbuterol. The smaller proportion of noradrenaline released acting on hypersensitive post-synaptic beta 1 adrenergic receptors would induce a greater effect (facilitation).

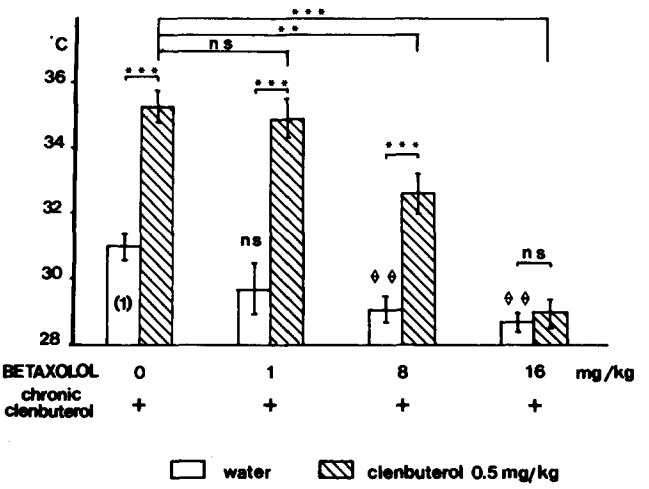

FIG. 3. Effect of betaxolol on the facilitation of clenbuterol-induced antagonism of reserpine hypothermia. Clenbuterol was chronically given: 0.25 mg·kg^{-1} IP, 19 injections on 12 days (for details, see the Method section) to all animals. Reserpine and betaxolol were administered respectively 4 hours and 15 minutes before the test-dose of clenbuterol (0.5 mg·kg^{-1}). Rectal temperature was measured 60 minutes after clenbuterol treatment. The values reported are the mean rectal temperature±S.E.M. for 7–8 mice in each group. Clear bars: water; hatched bars: clenbuterol 0.5 mg·kg^{-1}. [$F(7,51)=21.33$, $p<0.001$.] $^{**}p<0.01$, $^{***}p<0.001$. ◇: signification versus the group [1], ◇◇ $p<0.01$.

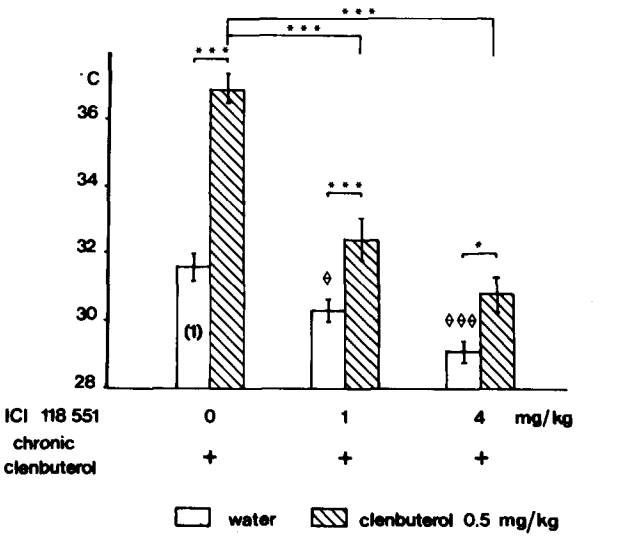

FIG. 4. Effect of ICI 118 551 on the facilitation of clenbuterol-induced antagonism of reserpine hypothermia. Clenbuterol was chronically administered: 0.25 mg·kg^{-1} IP, 19 injections on 12 days (for details, see the Method section) to all animals. Reserpine and ICI 118 551 were administered respectively 4 hours and 15 minutes before the test-dose of clenbuterol 0.5 mg·kg^{-1}. Rectal temperature was measured 60 minutes after clenbuterol treatment. The values reported are the mean rectal temperature±S.E.M. for 7 mice in each group. Clear bars: water; hatched bars: clenbuterol 0.5 mg·kg^{-1}. [$F(5,36)=37.34$, $p<0.001$.] $^{*}p<0.05$, $^{***}p<0.001$. ◇: signification versus the group [1], ◇ $p<0.05$; ◇◇◇ $p<0.001$.

In this hypothesized scheme the desensitized beta 2 receptors may respond to doses of ICI 118 551 inactive against acute clenbuterol; inversely the hypersensitized beta 1 receptors may respond to doses of betaxolol higher than those which are active against acute clenbuterol. This phenomenon is observed in our results.

In conclusion, the antagonism of reserpine-induced hypothermia depends primarily on a beta 1 mechanism. The action of clenbuterol on this test may be indirect. The facilitation of clenbuterol's effect after chronic treatment may involve a down-regulation of presynaptic beta 2 receptors and an hypersensitivity of post-synaptic beta 1 receptors.

REFERENCES

1. Bourin, M., M. Poncelet, R. Chermat and P. Simon. The value of the reserpine test in psychopharmacology. *Arzneimittelforsch* **33:** 1175–1176, 1983.
2. Dooley, D. H. and L. Hauser. Subchronic infusion of clenbuterol: a beta-adrenergic agonist, decreases the cerebellar beta-receptors. *Neurosci Lett* **36:** 96–97, 1983.
3. Engelhardt, G. Pharmacologisches Wirkungsprofil von N.A.B. 365 (clenbuterol) einen neuen broncholyticum mit einer selektiven wirkung auf die adrenergen beta 2 receptoren. *Arzneimittelforsch* **26:** 1404–1412, 1976.
4. Francès, H., A. J. Puech and P. Simon. Profil psychopharmacologique de l'isoprénaline et du salbutamol. *J Pharmacol* **9:** 25–34, 1978.
5. Francès, H. et P. Simon. Isoproterenol and psychopharmacological tests: antagonism by beta-adrenergic antagonists. *Pharmacol Res Commun* **10:** 211–217, 1978.
6. Francès, H., M. Poncelet, S. Danti, P. Goldschmidt and P. Simon. Psychopharmacological profile of clenbuterol. *Drug Dev Res* **3:** 349–356, 1983.
7. Francès, H., B. Diquet, P. Goldschmidt and P. Simon. Tolerance to or facilitation of pharmacological effects induced by chronic treatment with the beta-adrenergic stimulant clenbuterol. *J Neural Transm* **62:** 65–76, 1985.
8. Hawthorn, M. K. and K. J. Broadley. Evidence from use of neuronal uptake inhibition that β1-adrenoceptors, but not β2-adrenoceptors, are innervated. *J Pharm Pharmacol* **34:** 664–666, 1982.
9. Imbs, J. L., F. Miesch, J. Schwartz, J. Velly, G. Leclerc, A. Mann and C. G. Wermuth. A potent new β2 adrenoceptor blocking agent. *Br J Pharmacol* **60:** 357–363, 1977.
10. Manoury, P. Betaxolol: chemistry and biological profile in relation to its physico chemical properties. L.E.R.S. **1:** 13–19, 1983.
11. Minneman, K. P., R. N. Pittman and P. B. Molinoff. Beta-adrenergic receptor subtypes: Properties, distribution and regulation. *Annu Rev Neurosci* **4:** 419–461, 1981.
12. Pittman, R. N., K. P. Minneman and P. B. Molinoff. Alterations in beta 1 and beta 2 adrenergic receptor density in the cerebellum of aging rats. *J Neurochem* **35:** 273–275, 1980.
13. Ross, S. B. Antagonism of reserpine-induced hypothermia in mice by some beta-adrenoceptor agonist. *Acta Pharmacol Toxicol (Copenh)* **47:** 347–350, 1980.
14. Simon, P., Y. Lecrubier, R. Jouvent, A. J. Puech and D. Widlöcher. Beta-receptor stimulation in the treatment of depression. In: *Frontiers in Biochemical and Pharmacological Research in Depression,* Vol 39, edited by E. Costa *et al.* New York: Raven Press, 1984, pp. 273–282.