Pharmacology Biochemistry & Behavior, Vol. 26, pp. 11–15, 1987. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Beta-Adrenergic Agonists Reduce Spontaneous Motor Activity Through Either β1 or β2 Receptors

H. FRANCES,* N. RENWART,* S. DANTI,* R. CASH,†
R. RAISMAN† AND P. SIMON*

**Département de Pharmacologie, and †Département de Médecine Expérimentale*
Faculté de Médecine, Pitié-Salpêtrière 91
Boulevard de l'Hôpital, 75634 Paris Cédex 13, France

Received 13 June 1986

FRANCES, H., N. RENWART, S. DANTI, R. CASH, R. RAISMAN AND P. SIMON. *Beta-adrenergic agonists reduce spontaneous motor activity through either β1 or β2 receptors.* PHARMACOL BIOCHEM BEHAV **26**(1) 11–15, 1987.— In mice, the clenbuterol-induced decrease in spontaneous motor activity was antagonized by IPS-339 (β2 antagonist) but not by betaxolol (β1 antagonist), whereas the isoproterenol-induced decrease in spontaneous motor activity was completely antagonized by betaxolol and only partially by IPS-339. It can be concluded that the clenbuterol-induced decrease in spontaneous motor activity is of the β2-type, whereas that induced by isoproterenol is essentially of the β1 type. In addition, chronic treatment with clenbuterol induced a tachyphylaxis to the effect of clenbuterol but not of isoproterenol. After chronic administration of tricyclic antidepressants (imipramine and desipramine) the number of cortical β1 adrenergic receptors decreased without impairing the clenbuterol-induced decrease in spontaneous motor activity. We conclude that β2 adrenergic receptors mediate the clenbuterol-induced decrease in spontaneous motor activity and the tachyphylaxis to this effect after chronic treatment.

Spontaneous motor activity Clenbuterol Isoproterenol Beta-adrenergic receptors

THE beta-adrenergic agonists salbutamol, clenbuterol and isoproterenol decrease spontaneous motor activity in mice [3,5]. Clenbuterol and salbutamol are mainly beta 2 adrenergic agonists whereas isoproterenol also stimulates beta 1 adrenergic receptors. d-l-Propranolol antagonizes the decrease in spontaneous motor activity induced by isoproterenol [4] or by clenbuterol [7]. On the other hand, d-propranolol (which is devoid of beta-blocking effect) does not antagonize isoproterenol-induced decrease in motor activity. Therefore, these reductions in spontaneous motor activity seem to have a beta-adrenergic origin. Practolol (a beta 1 specific antagonist which does not penetrate easily into the brain) reduces the decrease in spontaneous motor activity induced by isoproterenol but not that induced by clenbuterol. This result leads to the conclusion of a beta 1 adrenergic component in the effect of isoproterenol but not in that of clenbuterol. The present study investigated more precisely the nature of the decreased spontaneous motor activities induced by different beta-adrenergic agonists.

METHOD

Animals

Male Swiss NMRI mice (20–24 g) and male Wistar rats (100–120 g) (CERJ, Genest, 53940 France) were used in all experiments. The animals were housed in groups of 10 with a 12 hr light-dark schedule in a room thermostatically maintained at 21±1°C. Food and water were freely available.

Drugs

Betaxolol hydrochloride (Lers. Synthelabo), IPS-339 (Metabio-Joulie), salbutamol sulfate (Glaxo), clenbuterol hydrochloride (Boehringer-Ingelheim), isoproterenol sulfate (Expandia), imipramine hydrochloride and desmethylimipramine hydrochloride (Ciba-Geigy) were dissolved in demineralized water.

All drugs were administered intraperitoneally in a volume of 0.25 ml/20 g body weight in mice and 0.5 ml/100 g body weight in rats.

Motor Activity

Spontaneous motor activity was measured in a photocell actimeter, one animal per cage [1]. In acute treatments, drugs were administered 30 min before the test. Antagonists were administered 45 min before testing.

Chronic Treatments

Chronic treatments were administered according to 3 different schedules. (1) Mice received clenbuterol IP at a dose

TABLE 1

ANTAGONISM OF CLENBUTEROL-INDUCED DECREASE IN SPONTANEOUS MOTOR ACTIVITY

mg·kg^{-1}		mg·kg^{-1}	Number of Light Beams Crossed M ± S.E.M.		%
−45 min Betaxolol		−30 min Clenbuterol			
0	+	0	226 ± 23		100
0	+	0.5	110 ± 9†	a	49
1	+	0.5	119 ± 9 N.S.	b	53
4	+	0.5	126 ± 19 N.S.	b	56
16	+	0.5	120 ± 20 N.S.	b	53
IPS-339		Clenbuterol			
0	+	0	323 ± 39		100
0	+	0.5	96 ± 12†	a	30
2	+	0.5	221 ± 31†	b	68
8	+	0.5	326 ± 64*	b	101

Beta-adrenergic antagonists and clenbuterol were administered 45 and 30 min respectively before the beginning of the test. Spontaneous motor activity was recorded during 30 min. Twelve mice per group.

Statistics: betaxolol + clenbuterol, $F(4,55)=7.59$, $p<0.001$; IPS-339 + clenbuterol, $F(3,44)=7.00$, $p<0.001$.

Significance: a vs. controls 0 + 0, b vs. controls 0 + clenbuterol. *$p<0.01$, †$p<0.001$.

TABLE 2

EFFECT OF BETA-ADRENERGIC ANTAGONISTS ON SPONTANEOUS MOTOR ACTIVITY

mg·kg^{-1}	Number of Light Beams Crossed M ± S.E.M.	%
Betaxolol		
0	226 ± 23	100
1	225 ± 22 N.S.	100
4	251 ± 26 N.S.	111
16	242 ± 35 N.S.	107
IPS-339		
0	323 ± 39	100
2	230 ± 28 N.S.	71
8	197 ± 21*	61

Betaxolol and IPS-339 were administered 45 min before the test. Spontaneous motor activity was recorded during 30 min. Twelve mice per group.

Statistics: betaxolol, $F(3,44)=0.36$, N.S.; IPS-339, $F(2,33)=4.52$, $p<0.05$.

*$p<0.05$.

of 0.25 mg·kg^{-1} twice a day (9 a.m., 6 p.m.). Controls received water. The experiments were performed approximately 6 hours after the last injection.

(2) Clenbuterol was dissolved in drinking water so as to furnish the same daily dose as in schedule 1. The concentration of clenbuterol in the drinking water was adjusted according to the mean weight of the mice (0.002 mg/ml of drinking water for a mouse of 20 g drinking 5 ml per day). The experiments were performed without preliminary withdrawal of the treatment.

(3) Rats received clenbuterol (10 mg·kg^{-1}) once a day, desipramine (10 mg·kg^{-1}) twice a day, imipramine (10 mg·kg^{-1}) twice a day or water IP. The animals were tested the day after the last treatment.

Binding Experiment

The density of beta-receptors in rat brain was measured with the beta-antagonist ^{3}H-dihydroalprenolol (^{3}H-DHA) (Amersham, 77 Ci/mmole).

After 8 days of clenbuterol treatment, the rats were killed and the cerebral cortex and cerebellum were dissected. The tissue was homogenized in a glass-teflon homogenizer in 33 volumes of cold buffer (50 mM Tris-HCl, pH 7.8, containing 120 mM NaCl, 5 mM KCl and 25 mM $MgCl_2$). The homogenates were washed twice by centrifugation at 35,000 g for 10 min. The final pellet was resuspended in the buffer at a concentration of 30 mg/ml (original tissue weight) and used immediately for the binding assay. The homogenate was incubated with 1.5 mM ^{3}H-DHA for 30 min at 25°C in a volume of 300 μl. Bound ligand was collected by rapid vacuum filtration through Whatman GF/C glass fiber filters, rinsed 3 times with 3 ml cold buffer, then dried and counted in a liquid scintillation spectrometer. All determinations were performed in duplicate. Specific binding was defined by displacement with propranolol (10 μM) and represented 70% of the total binding.

Statistical Analysis

The data were analysed by one way analysis of variance. Significant differences between groups were determined with Student's *t*-test.

RESULTS

Antagonism of Clenbuterol-Induced Decrease in Spontaneous Motor Activity

Betaxolol itself had no effect on spontaneous motor activity (Table 2). A slight decrease was observed in the presence of IPS-339 (Table 2). Both drugs antagonized the decrease in spontaneous motor activity (Table 1).

Antagonism of Isoproterenol-Induced Decrease in Spontaneous Motor Activity (Table 3)

The decrease in spontaneous motor activity induced by isoproterenol was completely antagonized by betaxolol at doses that were inactive against clenbuterol and was partly inhibited by IPS-339 at a dose which completely antagonized the effect of clenbuterol.

Repeated Treatment With Clenbuterol in Mice

First method (brief treatment). After 7 injections of clenbuterol (3.5 days) acute administration of clenbuterol or salbutamol no longer reduced spontaneous motor activity, but isoproterenol continued to be effective (Fig. 1A).

Second method (long lasting treatment). After 2 weeks of

TABLE 3

ANTAGONISM OF ISOPROTERENOL-INDUCED DECREASE IN SPONTANEOUS MOTOR ACTIVITY

	mg·kg⁻¹		Number of Light Beams Crossed M ± S.E.M.		%
−45 min Betaxolol		−30 min Isoproterenol			
0	+	0	250 ± 39		100
0	+	2	169 ± 12*	a	68
1	+	2	239 ± 18†	b	96
4	+	2	236 ± 26*	b	94
16	+	2	288 ± 35†	b	116
IPS-339		Isoproterenol			
0	+	0	288 ± 32		100
0	+	8	95 ± 8†	a	33
8	+	8	166 ± 17‡	b	58

Beta-adrenergic antagonists and isoproterenol were administered 45 and 30 min respectively before the beginning of the test. Spontaneous motor activity was registered for 30 min. Ten to twelve mice per group.

Statistics: betaxolol + isoproterenol, $F(4,55)=2.82$, $p<0.05$; IPS-339 + isoproterenol, $F(2,27)=20.46$, $p<0.001$.

Significance: a vs. controls 0 + 0, b vs. controls 0 + isoproterenol. *$p<0.05$, †$p<0.01$, ‡$p<0.001$.

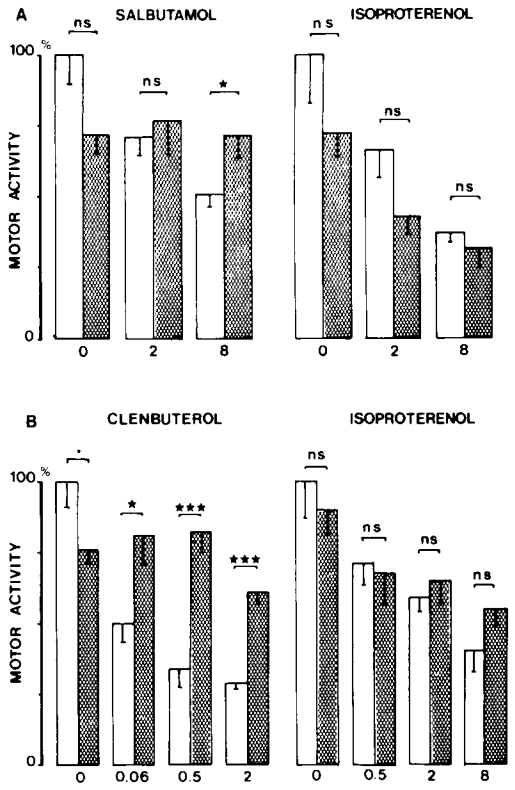

FIG. 1. Effect of chronic treatment with clenbuterol on the decrease in spontaneous motor activity induced by different beta-adrenergic agonists in mice. Chronic treatment: (A) Clenbuterol (0.25 mg·kg⁻¹) was administered IP twice a day during 3.5 days (=7 injections). The experiment was performed 6 hours after the last of repeated treatments. (B) Clenbuterol was given in drinking water in a concentration equal to the dose of 0.25 mg·kg⁻¹ (twice a day). The treatment lasted two weeks. Clear bars: controls receiving chronic water; cross-hatched bars: animals receiving chronic clenbuterol. Acute treatments were administered 30 min before the beginning of the tests. The drugs and doses are indicated on the figure. Motor activity was registered during 30 min. The mean number of light beams crossed was reported for 8–12 mice per group. Statistics: (A) Salbutamol, $F(5,66)=3.24$, $p<0.025$; isoproterenol, $F(5,65)=7.42$, $p<0.001$. (B) Clenbuterol, $F(7,69)=14.57$, $p<0.001$; isoproterenol, $F(7,69)=4.59$, $p<0.001$. ★$p<0.05$, ★★★$p<0.001$.

treatment, clenbuterol no longer reduced spontaneous motor activity whereas isoproterenol was still active (Fig. 1B).

Repeated Treatment With Clenbuterol, Desipramine or Imipramine in Rats (3rd Method)

After sub-chronic treatment with clenbuterol for 8 days, the reduction in spontaneous motor activity induced by acute administration of clenbuterol disappeared (Table 4).

After chronic treatment with desipramine (21 days), or imipramine (18 days), the decrease in spontaneous motor activity induced by acute clenbuterol persisted (Table 5).

Modification of the Number of Beta-Adrenergic Receptors in the Brain of Rats After Subchronic Treatment With Clenbuterol

The number of beta-adrenergic receptors in the cerebral cortex and cerebellum of rats was measured after a subchronic treatment of 8 days with clenbuterol. The number of beta-adrenergic receptors decreased by 29% in the cerebral cortex and by 70% in the cerebellum (Table 6).

DISCUSSION

The first part of this study showed that the decrease in spontaneous motor activity induced by clenbuterol is mediated by β2 receptors since it is blocked by the β2 antagonist IPS-339 [8] but not by the β1 antagonist betaxolol [9]. It is remarkable that IPS-339 which reduces spontaneous motor activity at a dose of 8 mg·kg⁻¹ also antagonizes the effect of clenbuterol. The isoproterenol-induced decrease in spontaneous motor activity was completely antagonized by betaxolol and partly antagonized by IPS-339. This is explained by its double property (β1 and β2) but underlines the more important participation of the β1 effect for isoproterenol.

In mice, a sub-chronic treatment of 7 administrations (3.5 days) with clenbuterol induced tachyphylaxis to the decrease in spontaneous motor activity induced by the same drug [7] but also to that induced by salbutamol, another β2 adrenergic stimulant. The effect of isoproterenol, however, was not antagonized by this treatment. This suggests that the tachyphylaxis reflects the specific down-regulation of β2 adrenergic receptors without modification of the β1 adrenergic receptors. This is in accordance with results demonstrating, after prolonged administration of clenbuterol in rats (minipumps), a selective decrease in the number of β2 adrenergic receptors in brain [2].

In preliminary investigations [6], it was observed that

TABLE 4

EFFECT OF REPEATED TREATMENTS WITH CLENBUTEROL ON THE DECREASE IN SPONTANEOUS MOTOR ACTIVITY INDUCED IN THE RAT BY THE SAME DRUG

Repeated Treatment Clenbuterol mg·kg^{-1}		Acute Treatment Clenbuterol mg·kg^{-1}	Number of Light Beams Crossed M ± S.E.M.		%
0 (×8)	+	0	333 ± 24		100
0 (×8)	+	0.5	90 ± 9*	a	27
10 (×8)	+	0	305 ± 21 N.S.	a	92
10 (×8)	+	0.5	299 ± 28*	b	90

The repeated treatment was administered once a day for 8 consecutive days. The experiment was performed on day 9. Rats received the acute treatment 30 min before the beginning of the test. Spontaneous motor activity was recorded during 30 min.

Twelve or thirteen rats per group.

Statistics: $F(3,46)=28.3$, $p<0.001$.

Significance: a vs. controls 0 + 0, b vs. control 0 + clenbuterol. *$p<0.001$.

TABLE 5

EFFECT OF REPEATED TREATMENT WITH IMIPRAMINE AND DESIPRAMINE ON THE DECREASE IN SPONTANEOUS MOTOR ACTIVITY INDUCED IN THE RAT BY CLENBUTEROL

Chronic mg·kg^{-1}		Acute mg·kg^{-1}	Number of Light Beams Crossed M ± S.E.M.		%
Imipramine		Clenbuterol			
0 (×36)	+	0	278 ± 24		100
0 (×36)	+	0.5	99 ± 17†	a	36
10 (×36)	+	0	214 ± 22*	a	77
10 (×36)	+	0.5	113 ± 16 N.S.	b	41
Desipramine		Clenbuterol			
0 (×42)	+	0	204 ± 14		100
0 (×42)	+	0.5	119 ± 9†	a	58
10 (×42)	+	0	167 ± 18 N.S.	a	82
10 (×42)	+	0.5	113 ± 14 N.S.	b	55

The repeated treatment was administered twice a day for 18 consecutive days (imipramine) or 21 consecutive days (desipramine). Experiments were performed on the day following the last treatment. Acute clenbuterol or water was given 30 min before the beginning of the test. Spontaneous motor activity was registered during 30 min. Ten or eleven rats per group.

Statistics: imipramine + clenbuterol, $F(3,37)=17.94$, $p<0.001$; desipramine + clenbuterol, $F(3,38)=8.79$, $p<0.001$.

Significance: a vs. controls 0 + 0, b vs. controls 0 + clenbuterol. *$p<0.05$, †$p<0.001$.

TABLE 6

MODIFICATION OF BRAIN NUMBER OF BETA-ADRENERGIC RECEPTORS AFTER REPEATED TREATMENTS WITH CLENBUTEROL

	^{3}H-DHA Binding fmol/mg Protein		Modification %
	Controls	Clenbuterol	
Cerebral Cortex	74.9 ± 4.0 (n=5)	57.9 ± 3.6* (n=5)	−29
Cerebellum	40.9 ± 2.1 (n=5)	12.4 ± 0.7† (n=5)	−70

Rats received clenbuterol 10 mg·kg^{-1} IP once a day (8 days); they were killed on the day following last treatment. n=number of measures (one rat for one measure).

Student's t-test: *$p<0.05$, †$p<0.01$.

chronic clenbuterol treatment induced tachyphylaxis to some of its effects after a short period of time and facilitation of other effects after a longer period (12 days). In the present study, even a two weeks treatment with clenbuterol did not modify the effect of isoproterenol, presumably because the $\beta 1$ receptors were not affected by the treatment.

The aim of the 3rd group of chronic experiments was to induce the well documented [1] down-regulation of cortical $\beta 1$ adrenergic receptors with tricyclic antidepressants.

In rats, the clenbuterol-induced decrease in spontaneous motor activity persisted after chronic treatments either with imipramine or desipramine (given in a time schedule known to reduce $\beta 1$ adrenergic receptors). Therefore, the cortical $\beta 1$ adrenergic receptors down-regulated by chronic antidepressant treatments are not involved in the effect of clenbuterol, although a tachyphylaxis to the effect of clenbuterol after chronic treatment with the same drug was observed.

After 8 days of sub-chronic treatment with clenbuterol, the number of beta-adrenergic receptors decreased in cortex and cerebellum of rats. The decrease was greater in the cerebellum than in the cerebral cortex and the ratio is in accordance with the repartition of the $\beta 2$ adrenergic receptors.

In conclusion, beta-adrenergic stimulants reduce spontaneous motor activity in rats and mice in two distinct ways, one involving $\beta 1$ and the other $\beta 2$ adrenergic receptors.

Tachyphylaxis to the decrease in spontaneous motor activity induced by a β2 agonist may be obtained without modifying that obtained with a β1 agonist. The down-regulation of the cortical β1 adrenergic receptors induced by chronic antidepressant treatment does not impair the decrease in spontaneous motor activity induced by clenbuterol.

REFERENCES

1. Boissier, J. R. and P. Simon. Action de la caféïne sur la motilité spontanée de la Souris. *Arch Int Pharmacodyn* **158:** 212–221, 1965.
2. Dooley, D. H. and L. Hauser. Subchronic infusion of clenbuterol: a beta-adrenergic agonist, decreases the cerebellar beta-receptors. *Neurosci Lett* **36:** 93–97, 1983.
3. Francès, H., A. J. Puech and P. Simon. Profil psychopharmacologique de l'isoprénaline et du salbutamol. *J Pharmacol* **9:** 25–34, 1978.
4. Francès, H. and P. Simon. Isoproterenol and psychopharmacological tests: antagonism by beta-adrenergic antagonists. *Pharmacol Res Commun* **10:** 211–217, 1978.
5. Francès, H., M. Poncelet, S. Danti, P. Goldschmidt and P. Simon. Psychopharmacological profile of clenbuterol. *Drug Dev Res* **3:** 349–356, 1983.
6. 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* **61:** 65–76, 1985.
7. Goldschmidt, P., H. Francès and P. Simon. Stimulation of beta-adrenergic receptors and spontaneous motor activity in mice. *Pharmacol Biochem Behav* **21:** 177–180, 1984.
8. Imbs, J. L., F. Miesch, J. Schwartz, J. Velly, G. Leclerc, A. Mann and C. G. Wermuth. A potent new B2 adrenoceptor blocking agent. *Br J Pharmacol* **60:** 357–363, 1977.
9. Manoury, P. Betaxolol: chemistry and biological profile in relation to its physico chemical properties. In: *Betaxolol and Other Beta 1-Adrenoceptor Antagonists. L.E.R.S. Monograph Series*, vol 1, edited by P. L. Morselli *et al.* New York: Raven Press, 1983, pp. 1–19.
10. Sulser, F. Antidepressant drug research: Its impact on neurobiology and psychobiology. In: *Typical and Atypical Antidepressants: Molecular Mechanisms*, edited by E. Costa and G. Racagni. New York: Raven Press, 1982, pp. 1–20.