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Disappearance of the Decrease in Biting Behavior Induced by Clenbuterol, A Beta-Adrenergic Agonist, After Chronic Administration

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FRANCES, H., S. DANTI, A.-J. PUECH AND P. SIMON. *Disappearance of the decrease in biting behavior induced by clenbuterol, a beta-adrenergic agonist, after chronic administration.* PHARMACOL BIOCHEM BEHAV **21**(2) 313–316, 1984.—The beta-adrenergic agonist clenbuterol decreased interest in food in starved mice, 30 minutes after administration. This effect disappeared after repeated treatment with clenbuterol (0.25 mg/kg, twice daily). Three chronic injections were sufficient to prevent the effect of an acute dose of clenbuterol (0.125 mg/kg) up to 45 hours after treatment.

Mice Biting behavior Beta-adrenergic agonist Chronic treatment Clenbuterol

ONE of the consequences of treatment with beta-adrenergic agonists is to decrease interest in food in food-deprived animals [3, 13, 14, 16, 21, 22, 26, 27]. Some effects of acute administration of beta-adrenergic agonist have been shown to disappear after chronic treatment. It has been reported that repeated administration of beta-adrenergic agonists induced "in vivo" a decreased response to acute treatment [1, 5, 18] and, in vitro, a down-regulation of beta-adrenergic receptor number and a decrease in beta-adrenergic stimulated cAMP formation [2, 7, 23, 25, 29]. Inversely hypersensitivity to adrenergic stimulation is observed after long term administration of beta-blocking drugs [4]. We wished, therefore, to determine whether repeated stimulation with beta-adrenergic agonists would decrease the effect of these substances on biting behavior in mice.

METHOD

The mice (male Swiss NMRI strain) weighed 20–25 g at the beginning of the experiments. Food and water were supplied ad lib. Food was withdrawn 24–27 hours before testing.

The "Tantalus" test, described by Dumeur *et al.* [9] was performed as follows. A food pellet of the type normally given to the mice was wrapped in a fine wire mesh in such a manner that the mice could not eat the pellet. This wire mesh wrapped pellet was then placed in the cage of a mouse deprived of food for one day. The number of times the animal bit the mesh was counted for 2 minutes after a 15 second habituation period. The test was always performed in the afternoon.

The test drug was administered by intraperitoneal route (IP) in a volume of 0.25 ml/20 g body weight, 30 minutes before the test session. The controls received demineralized water which was used as the solvent. Chronic treatment consisted of intraperitoneal injections of clenbuterol (0.25 mg/kg) twice daily (between 8:30 and 9:30 a.m.; between 5:00 and 6:30 p.m.). Controls received water. The animals were tested 6 hours after the last chronic injection, in order to determine the period of treatment necessary for "resistance" to develop. The disappearance of resistance was monitored as indicated in the text. Groups of ten mice, or multiples of 10, were used for each dose of the drug. Statistical analysis was performed using one-way analysis of variance followed by the Dunnett test [10], for Table 1 and Fig. 2. To determine the degree of resistance (Fig. 1), the method of Litchfield and Wilcoxon [24] was used to calculate the ED_{50}'s and their limits.

RESULTS

In the "Tantalus" test, the number of bites decreased but did not disappear following clenbuterol treatment. Residual bitings when the decrease was maximal was 40–30% of the control score. Test doses were the lowest dose producing the maximal effect (0.125 mg/kg) and one four times higher.

Development of Resistance (Table 1)

In animals pretreated once or repeatedly with water, clenbuterol (0.125 and 0.5 mg/kg) administered 30 minutes before the test decreased the number of bites. Because of widely varying results indicated by the S.E.M., this effect

TABLE 1

TANTALUS TEST: EFFECT OF ACUTE CLENBUTEROL TREATMENT ON THE NUMBER OF BITES AFTER CHRONIC TREATMENT WITH WATER OR CLENBUTEROL*

Chronic Treatment	Water			Clenbuterol 0.25 mg/kg		
Acute Clenbuterol mg/kg	0	0.125	0.5	0†	0.125	0.5
Number of Injections						
1	9.5±1.2	5.5±1.2 N.S.	5.7±1.1 N.S.	14.3±0.9‡	8.6±1.5§	10.5±1.2 N.S.
3	10.5±1.7	5.7±1.3 N.S.	5.3±1.5 N.S.	13.2±2.2 N.S.	12.8±1.3 N.S.	11.2±1.9 N.S.
5	12.9±1.1	5.4±1.5§	5.4±1.7§	12.6±1.6 N.S.	11.9±1.3 N.S.	9.1±1.5 N.S.
7	12.5±1.5	5.5±1.5§	5.1±1.0§	12.4±1.4 N.S.	13 ±1.7 N.S.	13.6±2.0 N.S.
15	11.9±0.8	4.4±1.2§	2.2±0.7§	14.9±2.2 N.S.	11.1±1.3 N.S.	11.0±1.5 N.S.
23	10.7±1.2	5.9±1.8 N.S.	4.5±0.9 N.S.	14.9±2.3 N.S.	15.7±2.1 N.S.	13.3±2.4 N.S.

*Mice were deprived of food for 24–27 hours. Acute doses, administered 30 minutes before test.

The number of times mesh wrapped food pellet bitten in 2 minutes after 15 seconds habituation: reported as mean ± s.e.m., n=10–20.

Last injection of chronic treatment (2 injections per day, 8:30–9:30 a.m.; 5:00–6:30 p.m.) given in the morning approximately 6 hours before performance of the test.

†Statistical significance calculated with respect to controls treated chronically and acutely with water. In the other columns significance with respect to controls receiving the same chronic treatment.

Statistics: one-way analysis of variance $F(5,112)=7.77$, $p<0.001$.

Dunnett's Test [10]: ‡$p<0.05$; §$p<0.01$.

was not always significant even when the number of bites decreased more than 50%. However, when animals were pretreated with clenbuterol, biting did not decrease except after a single pretreatment with clenbuterol 6 hours before acute treatment. In this case, the number of bites (8.6 and 10.5 with doses of 0.125 and 0.5 mg/kg respectively was similar to the score of controls receiving water only, either chronically and acutely (9.5 bites). In most cases the biting of controls receiving clenbuterol chronically was higher than that of controls receiving water. When comparisons between the two controls were performed for each experiment independently, a significant difference was found only after one pretreatment. If, however, the comparisons between the controls receiving clenbuterol chronically and these receiving water chronically was performed by combining the groups regardless of the number of pretreatments the following results were obtained. The mean number of bitings in controls pretreated with water was 10.83±0.54 (n=70) and in controls pretreated with clenbuterol 13.78±0.66 (n=69); using a one-way analysis of variance, the difference between the two values was highly significant ($p<0.001$).

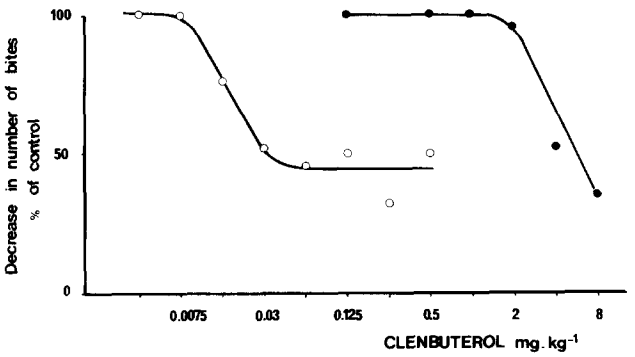

FIG. 1. Tantalus test: degree of resistance to acute administration of clenbuterol after chronic treatment. Chronic treatment consisted of 3 injections (IP) of water (○) or 0.25 mg/kg clenbuterol (●). The test was performed 6 hours after the last chronic injection. The mice had been deprived of food 24–27 hours before the experiment. The test doses progressed geometrically as multiples of two. Statistics: the doses decreasing the effect to 50% of the maximum (this maximum being 60–70% of the total effect) has been calculated using the test of Litchfield and Wilcoxon. The results are indicated in the text.

Degree of Resistance (Fig. 1)

Dose-response curves of the effect of acute administration of clenbuterol on the "Tantalus" test were established using mice rendered resistant to acute treatment by pretreatment (3 times) with clenbuterol compared to water (3 administrations) treated controls. Using the method described by Litchfield and Wilcoxon [24], we calculated the effective dose which reduced the number of bites to half (50%) maximal (ED_{50}) after transformation of the results to account for the fact that the reduction in biting is incomplete under these conditions. The ED_{50}'s and their fiducial limits for reducing the effect to half maximal was 0.03 mg/kg (0.02–0.04) for mice chronically treated with water, and 3.5 mg/kg (2.59–4.73) for mice chronically treated with clenbuterol.

Disappearance of Resistance (Fig. 2)

Resistance to clenbuterol could be detected 6 hours after the third pretreatment with clenbuterol (0.25 mg/kg). The effect was tested 21, 30, 45 and 54 hours after the third pretreatment. Clenbuterol (0.125 mg/kg) administered 30 minutes before the test was ineffective for 30 hours following pretreatment. Its effectiveness was partially restored after 45 hours and completely restored 54 hours after pretreatment.

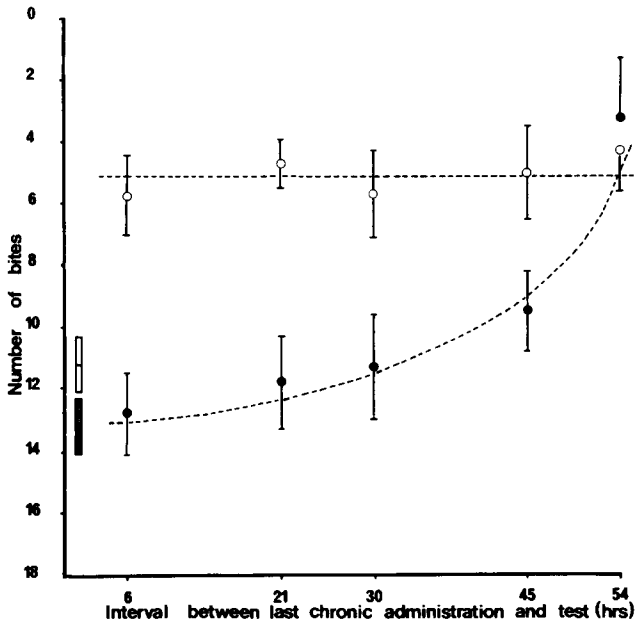

FIG. 2. Tantalus test: time course of disappearance of resistance to acute administration of clenbuterol after chronic treatment. Chronic treatment consisted of 3 injections (IP) of water (○) or 0.25 mg/kg clenbuterol (●). The test was performed 6, 21, 30, 45, 54 hours after the last chronic injection. The mice (10 per group) were deprived of food 24 to 27 hours before the experiment. The test dose was 0.125 mg/kg. The mean number of bitings was obtained from 10 mice per dose. Vertical bars represent the S.E.M. Open bar: controls (chronic and acute water); shaded bar: controls (chronic clenbuterol; acute water). Statistics: one-way analysis of variance, $F(9,87)=6.18$, $p<0.001$. Dunnett test [10]: $*p<0.05$; $**p<0.01$.

DISCUSSION

Clenbuterol, a beta-adrenergic agonist specific of beta 2 receptors [6,11], administered acutely decreases the biting behavior of starved mice. This behavior is not necessarily equivalent to feeding behavior. However, a parallelism is observed between the results of the "Tantalus" test and the ED_{50}'s obtained in a test of anorexia performed in rats with fenfluramine, d-amphetamine, amfepramone and clobenzorex (Dumeur *et al.*, personal communication). Moreover, the experiments described by Borsini *et al.* [3] demonstrated that the beta-adrenergic agonist salbutamol decreased food intake in rats. Salbutamol and isoproterenol also decreased biting behavior in the "Tantalus" test and clenbuterol is far more active than the other two beta-adrenergic agonists [13,14]. The greater activity of clenbuterol compared to salbutamol is probably explained by its greater lipophilicity which allows it to cross the blood brain barrier more readily [17,28]. This would suggest that clenbuterol acts on the CNS to produce biting behavior.

This study demonstrates that the clenbuterol induced decrease in biting behavior in starved mice disappears following chronic administration of this substance. Similar effects have been observed in other models. The development of resistance to the effects of beta-adrenergic agonists on cAMP and plasma glucose has been described in pregnant women treated with salbutamol [30]. Subchronic treatment (4 days) of female rats with isoprenaline reduced the relaxant effect of the drug on the uterus and decreased the density of beta-adrenergic receptors in myometrium membrane preparations [18]. It has been reported that intense use of beta-adrenergic agonists by asthmatic patients reduces their effectiveness on the respiratory system [5], but contradictory results have also been reported [19]. The increase in cardiac rhythm observed in man and dogs after rapid intravenous injection of isoprenaline is attenuated during intravenous injections lasting 15 to 210 minutes. The relaxant effect of isoprenaline and salbutamol on isolated fragments of rabbit thoracic aorta [15] could no longer be observed after prolonged exposure to the drugs. The decrease in exploratory behavior of rats after acute clenbuterol is reversed after chronic treatment [8].

The "resistance" in biting behavior induced by clenbuterol might be explained by a reduction in the number of beta-adrenergic receptors. This has been observed in rat cortex after peripheral administration of the drug [17]. The increase in biting behavior on control mice chronically treated with clenbuterol compared the control mice chronically treated with water may be another manifestation of the same phenomenon. If a beta-adrenergic stimulation occurs in satiety, as has been proposed [21], a reduction of this stimulation may reduce satiety and increase feeding behavior. When the number of beta-adrenergic receptors is decreased by a chronic treatment with a beta-stimulant a reduction in the action of endogenous noradrenaline may be expected. These results have two implications: firstly, the use of beta-adrenergic agonists as anorectics in man should be envisaged with prudence and only after long term study in animals. Secondly, the beta-adrenergic stimulant, salbutamol, has been shown to have, in animals, the psychopharmacological profile of an antidepressant drug [12] and has been used successfully in the treatment of depressive illness [20, 23, 31]. Its use for a prolonged period may lead to "resistance" to its antidepressant effects. This is now being studied in animals.

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