Biochemical Pharmacology, Vol. 38, No. 18, pp. 2957–2965, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00
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EFFECTS OF THE β_2-ADRENOCEPTOR AGONIST CLENBUTEROL ON TYROSINE AND TRYPTOPHAN IN PLASMA AND BRAIN OF THE RAT

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(*Received* 24 *October* 1988; *accepted* 12 *January* 1989)

Abstract—The β_2-adrenoceptor agonist, clenbuterol (initially 5 mg/kg), was found to significantly reduce plasma tyrosine and raise brain tryptophan levels ($P < 0.01$). By comparison, decreases in plasma tryptophan and increases in brain tyrosine were small and often nonsignificant. Amino acid levels measured in different brain regions revealed that the elevations were similar among the cerebellum, striatum, and cortex. These effects were partially blocked by propanolol but not by atenolol. The ED_{50} was estimated from dose–response curves to be about 0.05 mg/kg for both the decrease in plasma tyrosine and the increase in brain tryptophan. The effects of low doses of clenbuterol were prevented completely by propranolol. Peripheral organs displayed strikingly different patterns of change in amino acid concentrations. Only the spleen had any accumulation of tryptophan, but that was much less than in brain. In contrast, tyrosine and tryptophan were decreased in heart and unaltered in liver; tyrosine was decreased in lung. The elevation in brain tryptophan levels was attenuated by the β_2-antagonist, ICI 118,551, but not by the β_1-antagonist, betaxolol; but the reduction in plasma tyrosine was unaffected by either drug. The serotonin antagonist, methysergide, failed to block the effects of clenbuterol. We conclude that changes in amino acid concentrations produced by clenbuterol are mediated by β_2-adrenoceptor stimulation. Although the increases in brain tyrosine and tryptophan were similar to increases in the plasma ratios of these amino acids to the sum of the other large neutral amino acids competing for transport into the brain, the disparity between the effects of ICI 118,551 in brain and plasma suggests that clenbuterol may also have a direct action in brain to regulate levels of aromatic amino acids. Since clenbuterol has been purported to have an antidepressant effect and since other antidepressants also increase brain tryptophan, this may be a common feature of antidepressant drug action.

Recent work in our laboratory demonstrated that the antidepressant imipramine and the β_2-adrenoceptor agonist salbutamol have the common effects of reducing plasma tyrosine and elevating brain tryptophan concentrations [1]. The effects of imipramine are prevented or attenuated by propranolol, suggesting they are mediated, at least in part, by stimulating β-adrenoceptors. Since salbutamol has been reported to have antidepressant properties [2, 3], activating β-receptors may be an important feature of the antidepressant action of drugs. This idea is consistent with the fact that chronic treatment with virtually all antidepressants reduces the responsiveness of β-adrenergic receptors in rat brain, as measured by norepinephrine- and isoproterenol-induced accumulation of cyclic AMP [4], and reduces the density of β-adrenergic receptor binding sites [5].

The central actions of salbutamol may be hindered by the fact that the drug penetrates rather poorly into the brain, reaching a concentration of approximately 5% of that in the plasma [6]. Even these low amounts of drug may represent blood trapped in the brain rather than true brain levels, as suggested by the failure of salbutamol to reduce the binding of iodopindolol to β-adrenergic receptors in the brain [7]. However, these difficulties may be circumvented with clenbuterol, a β_2-adrenoceptor agonist that is more lipophilic than salbutamol and is believed to readily enter the brain, as evident by its dose-dependent inhibition of iodopindolol binding in brain [7]. Like salbutamol, clenbuterol has been claimed to display an antidepressant action [8].

Why salbutamol and clenbuterol might have antidepressant effects is somewhat puzzling, since they are selective for β_2-receptors, whereas classical antidepressants are generally thought to exert an action on β_1- but not β_2-receptors [9]. For example, chronic treatment with clenbuterol was found to preferentially reduce the density of β-receptors in the cerebellum [10], a brain region which contains predominately β_2-receptors [9], but this treatment had either small [10, 11] or no [12] effects on β-receptors in the cortex, which principally contains β_1-receptors [9]. Recently, however, Finnegan *et al.* [13] reported that long-term treatment with clenbuterol decreases β-adrenoceptor density in the frontal cortex by up to 60%, similar to the change found after long-term treatment with desmethylimipramine. Thus, whether or not clenbuterol has an influence on the density of β_1-receptors remains to be resolved. On the other hand, O'Donnell and Frazer [12] showed that clenbuterol causes an uncoupling of β-adrenoceptors from the N-protein in the rat cerebral cortex. This suggests that the drug may share with classical anti-

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depressants the ability to reduce the response to stimulating β_1-adrenoceptors, albeit by a different mechanism. Behavioral studies also suggest a common action of clenbuterol and the antidepressant desmethylimipramine by similarly reducing immobility in a forced swim test [13], an animal model widely used to screen for antidepressant activity.

In the present report, we compared the effects of clenbuterol on plasma and brain concentrations of the aromatic amino acids, tyrosine and tryptophan, with the effects we previously observed for imipramine and salbutamol [1]. In addition, we investigated the abilities of various β-antagonists to prevent these effects of clenbuterol.

MATERIALS AND METHODS

Animals and experimental procedures. Male Sprague–Dawley rats (200–250 g) were housed individually with a 12-hr light–dark cycle (lights on 6:00 a.m. to 6:00 p.m.) and with free access to food and water for 1 week before each experiment. All drugs were freshly prepared and administered intraperitoneally in a volume of 5 ml/kg, according to the schedules indicated in the Results. The animals were decapitated 90 min after the second injection. Trunk blood was collected into a plastic beaker containing 200 USP units of heparin and placed on ice. Brains were rapidly removed, blotted and immediately frozen on dry ice. They were stored at −50°. The blood samples were centrifuged at 500 *g* for 15 min, and the plasmas were stored at 4° until they were assayed on the next day.

Biochemical assays. Brain and plasma levels of tyrosine and tryptophan were assayed by high-performance liquid chromatography, as previously described for tyrosine [14] and modified for the simultaneous determination of tryptophan [1]. Plasma levels of phenylalanine were determined spectrophotofluorometrically [15]. Leucine, isoleucine and valine were isolated on Dowex 50 cation-exchange columns and were analyzed as the *t*-butyldimethylsilyl derivatives by gas chromatography–mass spectrometry, using the procedures of Schwenk *et al.* [16] as modified for chemical ionization (Edwards and Sorisio, manuscript in preparation).

Drugs. The following drugs were used: propranolol HCl and atenolol (Sigma Chemical Co., St Louis, MO), clenbuterol HCl (Boehringer Ingelheim, St Joseph, MO), methysergide maleate (Sandoz, East Hanover, NJ), ICI 118,551 (Imperial Chemical Industries, Macclesfield, U.K.) and betaxolol HCl (L.E.R. Synthelabo, Paris, France).

Statistics. Data were analyzed by analysis of variance (ANOVA) followed by a Newman–Keuls multiple comparison test, unless otherwise indicated.

RESULTS

Effects of 5 mg/kg of clenbuterol on tyrosine and tryptophan concentrations in plasma and specific brain regions. In the first experiment, weight-matched groups of rats were injected i.p. with saline, 15 mg/kg propranolol (a lipophilic, nonselective β-antagonist which readily enters the brain) or 15 mg/kg atenolol (a weakly lipophilic, β_1-selective antagonist which does not penetrate easily into the brain). Twenty minutes later each rat received a second injection, either saline or 5 mg/kg clenbuterol. The rats were killed 90 min later, the brains were removed rapidly, and the cerebella, striata and cerebral cortices were dissected.

As shown in Fig. 1, clenbuterol increased tyrosine concentrations in all three brain regions, but the increase was statistically significant only in the cortex (to 119% of control). Propranolol appeared to prevent any changes in tyrosine levels in these brain regions: tyrosine following clenbuterol was 100, 112 and 98% of control in the cerebellum, striatum and cerebral cortex respectively. Atenolol, on the other hand, did not block the rise in tyrosine but appeared to actually enhance these increases.

Clenbuterol elevated tryptophan concentrations by similar amounts in each brain region: to 177% in the cerebellum, to 165% in the striatum and to 170% in the cortex respectively (each $P < 0.01$). Propranolol attenuated these increases, to 138, 130 and 125% respectively. Atenolol not only failed to prevent the increases in tryptophan but, as for tyrosine, may have enhanced them. In each region, the tryptophan levels were higher after treatment with atenolol + clenbuterol than with saline + clenbuterol.

The plasma concentration of tyrosine was decreased by clenbuterol to 64% of control ($P < 0.05$). This decrease was partially prevented by pretreatment with propranolol. Clenbuterol lowered plasma tyrosine nonsignificantly to 80% compared to animals treated with propranolol alone. However, because propranolol by itself caused a small but nonsignificant decrease in plasma tyrosine, levels in the group that received both clenbuterol and propranolol were statistically lower than the saline–saline group. On the other hand, the decrease in atenolol-pretreated rats was to 73%, which was similar to that observed in the saline-pretreated animals, although it did not quite reach statistical significance. However, as in the case of the propranolol–clenbuterol group, plasma tyrosine was statistically reduced as compared to the saline–saline group. Plasma tryptophan levels were reduced slightly but nonsignificantly by clenbuterol, whether or not the animals were pretreated with a β-antagonist. These levels were lowered to 82, 78 and 85% of the corresponding control in saline, propranolol and atenolol pretreated groups, respectively.

Dose–response for the effects of clenbuterol and antagonism by propranolol. To better characterize the effects of clenbuterol, a dose–response study was carried out. Since propranolol did not block completely the effects of 5 mg/kg of clenbuterol, it was of interest to find out whether the effects of a lower dose of clenbuterol would be antagonized more effectively by a β-blocker or whether the effects of clenbuterol are due only partly to β-receptor activation. Moreover, a comparison of the dose–response curves for various biochemical changes might indicate whether they were caused by common or distinct mechanisms.

Since all rats were killed between 2:00 p.m. and 3:30 p.m. to minimize circadian changes, it was necessary to combine data from four separate experi-

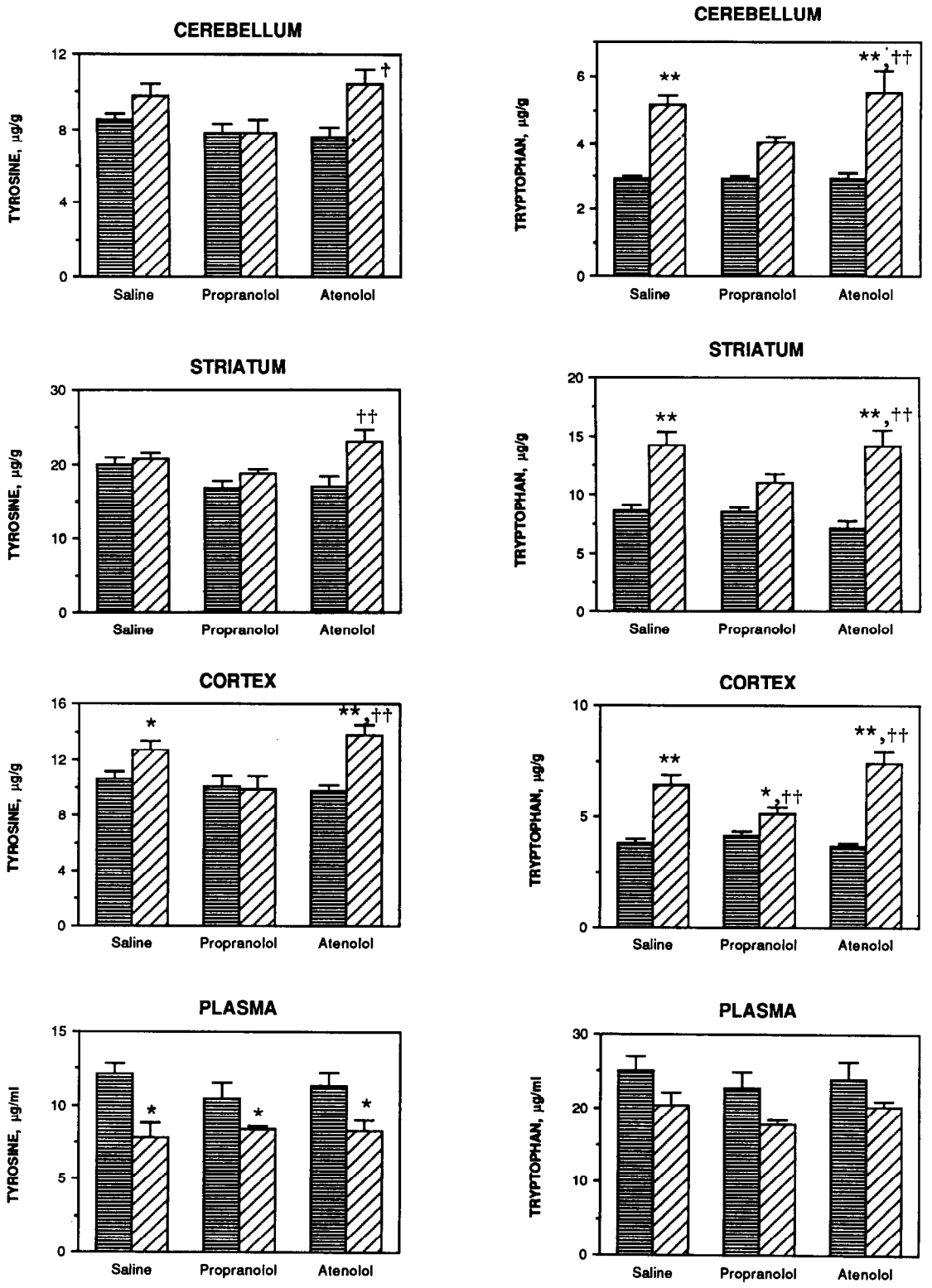

Fig. 1. Effects of clenbuterol on tyrosine and tryptophan in specific brain regions and in plasma. Rats were injected with saline or propranolol (15 mg/kg), or atenolol (15 mg/kg), and 20 min later with either saline or clenbuterol (5 mg/kg). The rats were killed 90 min after the second injection. Values are means ± SE (N = 5). Key: (*) $P < 0.05$; and (**) $P < 0.01$, vs saline–saline control group, Newman–Keuls multiple comparison tests; (†) $P < 0.05$ and (††) $P < 0.01$, vs the corresponding control group receiving saline in place of propranolol or atenolol, Newman–Keuls multiple comparison tests.

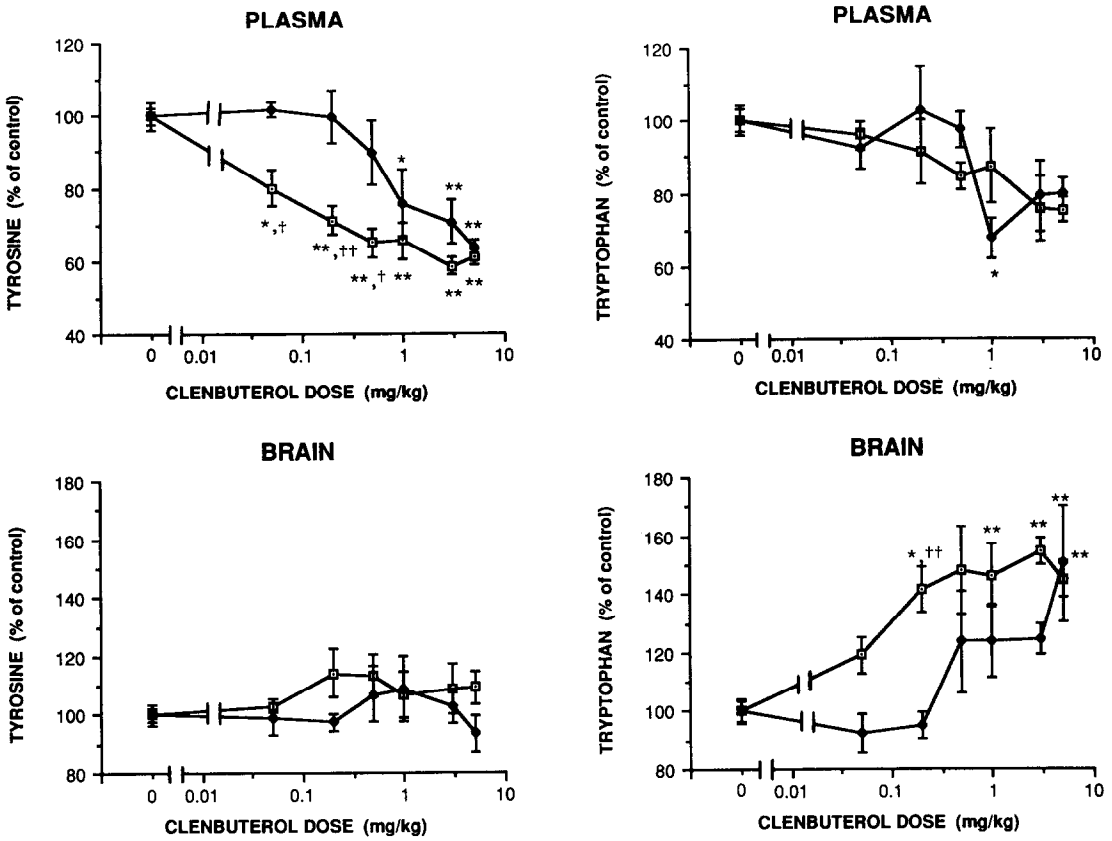

Fig. 2. Dose–response relationships for the effects of clenbuterol on plasma and brain concentrations of tyrosine and tryptophan with (–●–) and without (–□–) propranolol pretreatment. Rats were injected i.p. with either saline or propranolol (15 mg/kg) and 20 min later with saline or a dose of clenbuterol over the range of 0.05 to 5 mg/kg. The animals were killed 90 min after the second injection. The data were combined from four experiments, expressed as the percent of control for each. The control values (mean ± SE) for the saline–saline group in the separate experiments were as follows: plasma tyrosine, 17.47 ± 1.21, 16.46 ± 0.98, 14.26 ± 0.42, 10.84 ± 0.60 μg/ml; plasma tryptophan, 15.32 ± 1.45, 14.62 ± 0.80, 16.01 ± 0.96, 17.12 ± 1.06 μg/ml; brain tyrosine, 15.30 ± 0.57, 13.18 ± 0.48, 11.62 ± 0.50, 10.70 ± 0.77 μg/g; and brain tryptophan, 4.97 ± 0.34, 3.00 ± 0.24, 2.98 ± 0.33, 4.00 ± 0.22 μg/g. The data were analyzed by a one-way ANOVA [plasma tyrosine, $F = 13.831$, $P < 0.001$; plasma tryptophan, $F = 3.830$, $P < 0.001$; brain tryosine, not significant (NS); and brain tryptophan, $F = 8.196$, $P < 0.001$] followed by a Newman–Keuls multiple comparison test. Key: (*) $P < 0.05$, and (**) $P < 0.01$, compared to the corresponding control group receiving saline in place of clenbuterol; (†) $P < 0.05$, (‡) $P < 0.01$, compared to the corresponding control group receiving saline in place of propranolol.

ments. As shown in Fig. 2, significant declines in plasma tyrosine levels were observed with all doses of clenbuterol (0.05–5 mg/kg). Propranolol completely antagonized the effects of low doses of clenbuterol but failed to overcome the effects of the high doses.

As in Fig. 1, high doses of clenbuterol non-significantly reduced plasma tryptophan levels. Since the decreases were not statistically significant, it is impossible to say whether propranolol prevented them. Brain tryptophan levels were elevated significantly by all except the lowest dose of clenbuterol. Propranolol appeared to completely block the effects of the low doses, and the saline- and propranolol-pretreated groups were statistically different for the 0.2 mg/kg dose. Brain tyrosine concentrations were increased slightly by all doses of clenbuterol, but these changes were not statistically significant by one-way ANOVA.

Effects of clenbuterol on tyrosine and tryptophan levels in peripheral organs. The increases in amino acid concentrations in the brain with a concomitant decrease in plasma could be due to an increase in their transport across the blood–brain barrier. Alternatively, there might be a general cellular uptake of amino acid resulting in an increase in levels in other tissues as well. To distinguish between these two possibilities, tyrosine and tryptophan concentrations were assayed in heart, lung, spleen and liver of the same animals as shown in Fig. 2 for brain and plasma. The results show that, of the four peripheral organs, only the spleen had any accumulation of these amino acids following clenbuterol treatment (Fig. 3). In contrast, both amino acids were reduced in heart, as they were in plasma. Tyrosine was reduced in lung, but neither amino acid was changed in liver. Surprisingly, the effect on tyrosine in lung was evident only at the 1 mg/kg dose and not at the higher doses.

Effects of the selective β_1- and β_2-antagonists, betaxolol and ICI 118,551. Another experiment was car-

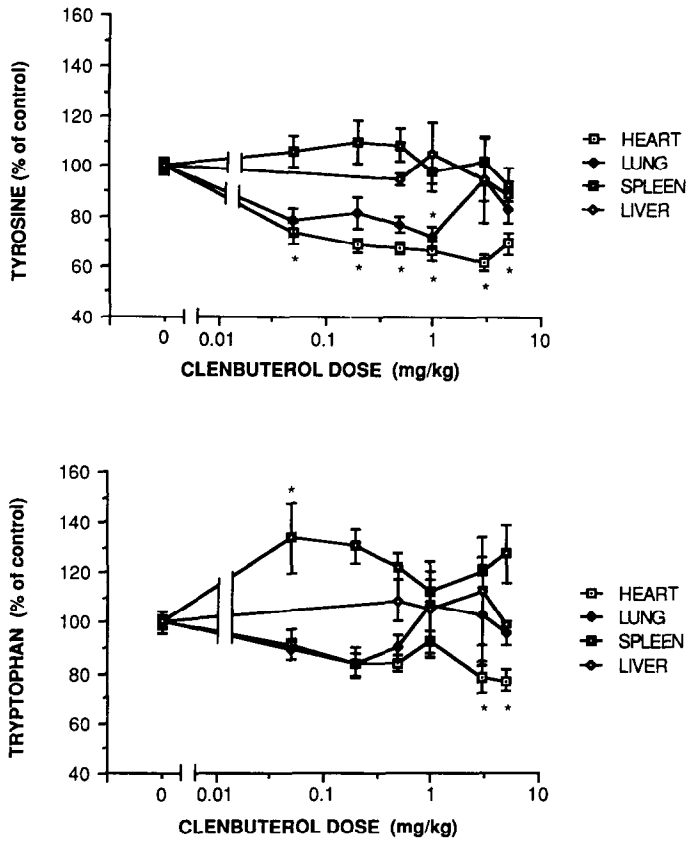

Fig. 3. Dose–response relationships for the effects of clenbuterol on tyrosine and tryptophan concentrations in heart, lung, spleen and liver. The data were combined for the four experiments included in Fig. 2 (three experiments for liver) and are expressed as the percent of control. The control values (mean ± SE) for the saline–saline group in the separate experiments were as follows: heart tyrosine, 23.77 ± 1.73, 20.79 ± 0.88, 21.19 ± 0.53, 15.38 ± 0.81 μg/g; lung tyrosine, 38.61 ± 3.06, 31.93 ± 2.12, 31.02 ± 1.98, 20.98 ± 1.02 μg/g; spleen tyrosine, 53.53 ± 5.20, 58.63 ± 2.94, 53.47 ± 3.22, 31.22 ± 1.93 μg/g; and liver tyrosine, 47.20 ± 1.93, 34.41 ± 2.75 and 46.14 ± 6.45 μg/g; heart tryptophan, 8.70 ± 0.73, 7.36 ± 0.55, 8.07 ± 0.57, 7.37 ± 0.47 μg/g; lung tryptophan, 10.15 ± 0.80, 10.64 ± 0.94, 10.31 ± 0.62, 9.13 ± 0.36 μg/g; spleen tryptophan, 10.87 ± 0.82, 15.61 ± 1.05, 10.82 ± 0.56, 8.89 ± 1.01 μg/g; and liver tryptophan, 10.07 ± 0.92, 6.34 ± 0.38 and 17.53 ± 3.9 μg/g. The data were analyzed by a one-way ANOVA (tyrosine: heart, $F = 26.848$, $P < 0.001$; lung, $F = 4.524$, $P < 0.001$; spleen and liver, NS; tryptophan: heart, $F = 5.117$, $P < 0.001$; lung, NS; spleen, $F = 3.407$, $P < 0.01$; liver, NS) followed by a Newman–Keuls multiple comparison test. Key: (*) $P < 0.01$, compared to the corresponding control group receiving saline in place of clenbuterol.

ried out using antagonists selective for β_1- or β_2-receptors, betaxolol (2 mg/kg) and ICI 118,551 (1 mg/kg), respectively, in place of propranolol. The results (Fig. 4) show that ICI 118,551 but not betaxolol attenuated the elevation in brain tryptophan caused by 0.5 mg/kg of clenbuterol. A similar pattern may have occurred for brain tyrosine, but the 13% increase did not reach statistical significance. In contrast, the effect of clenbuterol on plasma tyrosine levels was not blocked by either antagonist. Thus, clenbuterol reduced plasma tyrosine levels to 70% of control in the saline-pretreated group ($P < 0.01$), to 67% in the ICI 118,551-pretreated group ($P < 0.01$) and to 76% in the betaxolol-pretreated group ($P < 0.01$). This dose of clenbuterol did not significantly lower plasma tryptophan levels.

Effects of clenbuterol after pretreatment with methysergide. Since clenbuterol is known to stimulate serotonergic in addition to β-receptor function [17, 18] and propranolol has an antiserotonin action [19, 20], it is possible that some of the effects of clenbuterol that we have observed were mediated by an action on serotonin receptors rather than on β-receptors. This possibility was tested by an experiment in which rats were pretreated with 2 mg/kg methysergide, a serotonin antagonist [21], 30 min prior to clenbuterol. Three additional groups (N = 5–7/group) of rats received saline in place of methysergide (clenbuterol only), saline in place of clenbuterol (methysergide only) or saline in place of both drugs (saline–saline). All animals were killed 90 min after the second injection.

The administration of clenbuterol alone reduced plasma tyrosine and tryptophan levels to 64 and 72%, respectively, and elevated brain tyrosine to 117% and brain tryptophan to 192%. In methy-

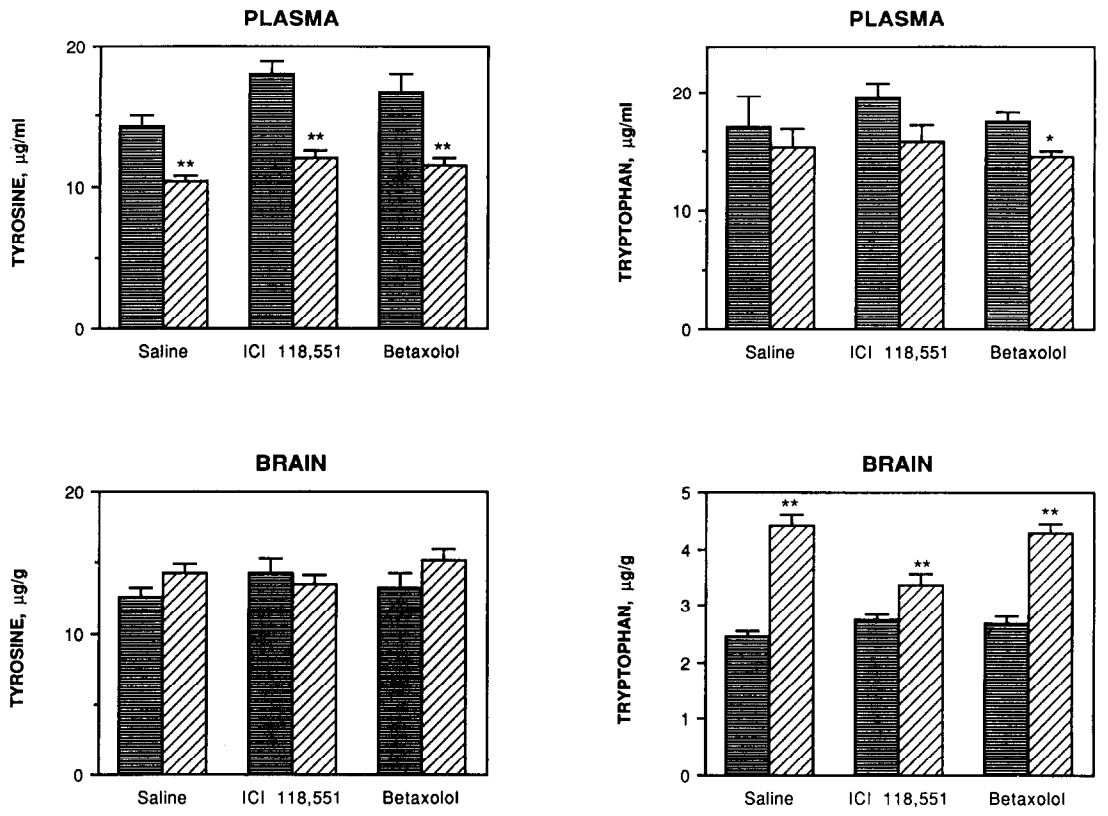

Fig. 4. Effects of the selective antagonists, ICI 118,551 and betaxolol, on the action of 0.5 mg/kg clenbuterol. Rats were pretreated with 1 mg/kg of the β_2-antagonist ICI 118,551, 2 mg/kg of the β_1-antagonist betaxolol or saline 20 min before saline (▤) or 0.5 mg/kg clenbuterol (▨). All animals were killed 90 min after the second injection. The data were analyzed by one-way ANOVA (plasma tyrosine, $F = 14.90$, $P < 0.001$; plasma tryptophan, $F = 3.964$, $P < 0.01$; brain tyrosine, NS; and brain tryptophan, $F = 32.76$, $P < 0.001$) followed by Newman–Keuls multiple comparison test. Key: (*) $P < 0.05$, and (**) $P < 0.01$, compared to the corresponding saline-pretreated control group.

sergide-pretreated rats, clenbuterol lowered plasma tyrosine to 62%, lowered plasma tryptophan to 63%, increased brain tyrosine to 124% and increased brain tryptophan to 199%. When these groups were statistically compared by two-way ANOVA, clenbuterol was found to produce significant increases in brain tyrosine ($F = 18.61$; $P < 0.001$) and tryptophan ($F = 53.24$; $P < 0.001$) and decreases in plasma tyrosine ($F = 34.36$; $P < 0.001$) and tryptophan ($F = 28.03$; $P < 0.001$). Methysergide had no effect on tyrosine levels and caused only a small, but statistically significant 13% reduction in tryptophan levels in plasma ($F = 7.06$; $P < 0.025$) and a 19% increase in brain ($F = 5.12$; $P < 0.05$). There was no significant interaction between clenbuterol and methysergide.

Effects of clenbuterol on other large neutral amino acids. The transport of each large neutral amino acid into the brain has been shown to correlate with the serum concentration ratio of that amino acid to the sum of the other large neutral amino acids [22]. Therefore, we examined the influence of clenbuterol on the plasma concentrations of large neutral amino acids other than tyrosine and tryptophan. The results indicate that 0.2 mg/kg of clenbuterol decreased plasma phenylalanine to about the same extent as tyrosine but reduced the three branched-chain amino acids, leucine, isoleucine and valine, to an even greater extent, to approximately 50% (Table 1). Consistent with the above results (Figs 1–3), plasma tryptophan was lowered slightly but nonsignificantly by this dose of clenbuterol. The ratio of tryptophan to the sum of the competing aromatic and branched-chain amino acids (expressed in molar concentrations) was 0.125 in the control group and 0.210 in the clenbuterol-treated group. Thus, clenbuterol increased this ratio by 68%, which completely accounts for the 42% rise in the brain tryptophan observed in the same experiment. The corresponding ratios of tyrosine to the sum of the competing amino acids were 0.086 and 0.107 in the control and drug-treated groups respectively. Based on these results, a 24% rise in brain tyrosine would be predicted, which is in reasonable agreement with the 14% actually observed.

DISCUSSION

This study demonstrates that clenbuterol alters the

Table 1. Effects of clenbuterol (0.2 mg/kg) on the plasma concentrations of various large neutral amino acids

	Plasma amino acid concentration ng/ml		nmol/ml	
Amino acid	Saline	Clenbuterol	Saline	Clenbuterol
Tyrosine	10.84 ± 0.60	7.71 ± 0.43* (71%)	59.9	42.6
Tryptophan	17.12 ± 1.06	15.60 ± 1.51 (91%)	83.9	76.5
Phenylalanine	15.24 ± 0.96	9.45 ± 0.79† (62%)	92.4	57.3
Leucine	26.45 ± 2.28	14.45 ± 1.59* (55%)	201.9	110.3
Isoleucine	17.22 ± 1.44	8.11 ± 1.03† (47%)	131.5	61.9
Valine	21.80 ± 1.84	10.72 ± 1.13† (49%)	186.3	91.6
$\frac{\text{Tryptophan}}{\Sigma\text{Tyr+Phe+Leu+Ile+Val}}$			0.125	0.210
$\frac{\text{Tyrosine}}{\Sigma\text{Trp+Phe+Leu+Ile+Val}}$			0.086	0.107

Values are means ± SE (N = 4–5).
*, † Significantly different from saline: * $P < 0.01$, and † $P < 0.005$ (Student's two-tailed t-test).

concentrations of tyrosine and tryptophan in brain and plasma. The decreases in plasma tyrosine and the increases in brain tryptophan concentrations were consistent with the results we previously found for salbutamol [1], but the effects of clenbuterol were larger even at one-half the dose. In addition, clenbuterol appeared to reduce plasma tryptophan to about 70–90% of control and increase brain tyrosine by about 20%; but these changes did not always reach statistical significance.

The ED_{50} for both the decrease in plasma tyrosine and the increase in brain tryptophan was estimated to be approximately 0.05 mg/kg. This dose is very similar to the ED_{50} reported for clenbuterol to control differential operant responding in rats (ED_{50} = 0.03 mg/kg) [23], but is far lower than the doses that have generally been employed for examining biochemical effects of the drug. For example, down-regulation of cortical β-adrenoceptors was reported when clenbuterol was administered 5 mg/kg twice daily for 14 days [11] or 5–35 mg/kg once daily for 7 days [13].

There are several possible mechanisms by which clenbuterol may decrease plasma and increase brain tyrosine and tryptophan levels. First, the drug may generally stimulate intracellular amino acid uptake. This, however, appears unlikely, since clenbuterol decreased or did not change tyrosine and tryptophan levels in three of the four organs studied (i.e. liver, heart and lung) and increased them only slightly in the spleen (Fig. 2).

Tyrosine and tryptophan could increase in brain secondarily to increases in transport across the blood–brain barrier. Such a possibility would explain: (1) why salbutamol [1] and isoproterenol [24] affect brain amino acid levels even though neither readily crosses the blood–brain barrier; (2) why nadolol, a β-blocker that does not easily penetrate into the brain [25], prevents the effects of clenbuterol in brain (D. J. Edwards and D. A. Sorisio, unpublished observation); and (3) why clenbuterol produces similar effects on the amino acid levels in the cerebellum, a region rich in β_2-receptors, as in the striatum and cerebral cortex, areas containing predominately β_1-receptors.

Since tyrosine and tryptophan compete with other large neutral amino acids for their transport across the blood–brain barrier [22], brain tyrosine and tryptophan uptake would increase if their ratios to the other large neutral amino acids were increased in plasma. Studies of diet-induced changes in brain levels of large neutral amino acids indicate that the changes in each correlate very well with the plasma ratio of that amino acid to the sum of the others [22]. The data in Table 1 are consistent with a similar mechanism for the effects of clenbuterol, since the increases in brain tyrosine and tryptophan levels were similar to the increases in their plasma ratios. On the other hand, evidence that clenbuterol can alter brain amino acid concentrations independent of any changes in plasma levels is provided by the finding that ICI 118,551 attenutated the clenbuterol-induced elevation of brain tryptophan and perhaps of tyrosine (although the increase in tyrosine was nonsignificant) without having any influence on clenbuterol-induced changes in plasma levels (Fig. 4). Similar results were also noted for nadolol (D. J. Edwards and D. A. Sorisio, unpublished observation).

Eriksson and Carlsson [26] recently reported that isoproterenol decreases the plasma levels of each of the large neutral amino acids to a similar extent, whereas the brain level of each (except for leucine and isoleucine) is increased. Based on these findings, they concluded that "the total concentration of large neutral amino acids can obviously not be explained

by the competition between these amino acids for the carrier-mediated transport into the brain." They proposed that the influence of isoproterenol might be mediated by β-receptors directly affecting the brain transport system. We cannot rule out the possibility that clenbuterol acts by such a mechanism, but we did not measure brain levels of phenylalanine and the branched-chain amino acids.

The low ED_{50} for clenbuterol combined with the ability of the β_2-selective antagonist, ICI 118,551, but not the β_1-selective antagonists, atenolol (Fig. 1) and betaxolol (Fig. 4), to block the effects of clenbuterol suggest that the changes in brain tyrosine and tryptophan levels are mediated by β_2-adrenoceptors. Moreover, although clenbuterol has affinity for both β_1- and β_2-receptors in brain [27], the drug acts functionally as an antagonist at β_1-receptors [28]. Therefore, β_1-adrenoceptors do not appear to have a role in clenbuterol altering brain amino acid concentrations. ICI 118,551 did not block the effects on plasma levels, but we cannot exclude the possibility that this also involves β_2-receptors. Perhaps receptors regulating peripheral amino acid levels are more sensitive to clenbuterol or are exposed to a higher drug concentration, so that a higher dose of the antagonist would be needed.

It is possible that disparities among the effects of different antagonists may be related to their ability to act as serotonin antagonists [19, 20]. Since salbutamol [17] and clenbuterol [18] stimulate serotonin receptors, effects mediated by this mechanism would be expected to be blocked by propranolol, which is a potent serotonin antagonist, but not by atenolol, which is not [19, 20]. That methysergide, a serotonin antagonist [21], was unable to prevent the effects of clenbuterol on both brain and plasma amino acid levels would argue against this possibility. However, since methysergide does not have equal potency for all serotonin receptor subtypes, further studies are needed with other serotonin antagonists.

The increase in brain tryptophan may be particularly relevant to the antidepressant property of clenbuterol [8], since imipramine and other typical and atypical antidepressants have been reported previously to do the same [1, 29]. Presumably, an increase in brain tryptophan would enhance brain serotonin synthesis as a common mechanism for antidepressant effects. This could explain why salbutamol may have an antidepressant action, despite penetrating poorly into the brain, since the drug could act in the periphery to increase brain tryptophan uptake.

Even if clenbuterol and classical antidepressants act in common to increase brain tryptophan levels, they may do so by different mechanism. The present studies suggest that the biochemical changes produced by clenbuterol are mediated by the stimulation of β_2-adrenoceptors. However, imipramine is believed to selectively affect β_1-adrenoceptors [9]. Interestingly, clenbuterol has been reported to reduce spontaneous motor activity in mice through either β_1- or β_2-receptors [30]. Further studies are needed to determine whether both receptor subtypes are involved in the antidepressant action of drugs.

Acknowledgements—This work was supported by NIMH Grant MH-28340. We thank Boehringer Ingelheim, Sandoz, ICI and L.E.R. Synthelabo for gifts of clenbuterol, methysergide, ICI 118,551 and betaxolol respectively.

REFERENCES

1. Edwards DJ and Sorisio DA, Effects of imipramine on tyrosine and tryptophan are mediated by β-adrenoceptor stimulation *Life Sci* **42**: 853–862, 1988.
2. Belmaker RH, Lerer B and Zohar J, Salbutamol treatment of depression. In: *Typical and Atypical Antidepressants: Clinical Practice* (Eds. Costa E and Racagni G), pp. 181–193. Raven Press, New York, 1982.
3. Lecrubier Y, Puech AJ, Jouvent R, Simon P and Widlocher D, A beta-adrenergic stimulant (salbutamol) versus clomipramine in depression: a controlled study. *Br J Psychiatry* **136**: 354–358, 1980.
4. Vetulani J and Sulser F, Action of various antidepressant treatments reduces reactivity of noradrenergic cyclic AMP generating system in limbic forebrain. *Nature (Lond)* **257**: 495, 1975.
5. Banerjee SP, Kung LS, Riggin SJ and Chanda SK, Development of β-adrenergic receptor subsensitivity by antidepressants. *Nature (Lond)* **268**: 455–456, 1977.
6. Caccia S and Fong MH, Kinetics and distribution of the β-adrenergic agonist salbutamol in rat brain. *J Pharm Pharmacol* **36**: 200–202, 1984.
7. Conway PG, Tejani-Butt S and Brunswick DJ, Interaction of *beta* adrenergic agonists and antagonists with brain *beta* adrenergic receptors *in vivo*. *J Pharmacol Exp Ther* **241**: 755–762, 1987.
8. Simon P, Lecrubier Y, Jouvent R, Puech A and Widlocher D, Beta-receptor stimulation in the treatment of depression. In: *Frontiers in Biochemical and Pharmacological Research in Depression* (Eds. Usdin E, Asberg M, Bertilsson L and Sjoqvist F), pp. 293–299. Raven Press, New York, 1984.
9. Minneman KP, Wolfe BB, Pittman RN and Molinoff PB, β-Adrenergic receptor subtypes in rat brain. In: *Molecular Pharmacology of Neurotransmitter Receptors* (Eds. Segawa T, Yamamura K and Kuriyama K), pp. 73–81. Raven Press, New York, 1983.
10. Dooley DJ and Hauser KL, Subchronic infusion of clenbuterol, a *beta*-adrenergic agonist, decreases the cerebellar beta receptor. *Neurosci Lett* **36**: 93–97, 1983.
11. Nimgaonkar VL, Goodwin GM, Davies CL and Green AR, Down-regulation of β-adrenoceptors in rat cortex by repeated administration of desipramine, electroconvulsive shock and clenbuterol requires 5-HT neurones but not 5-HT. *Neuropharmacology* **24**: 279–283, 1985.
12. O'Donnell JM and Frazer A, Effects of clenbuterol and antidepressant drugs on *beta* adrenergic receptor/N-protein coupling in the cerebral cortex of the rat. *J Pharmacol Exp Ther* **234**: 30–36, 1985.
13. Finnegan KT, Terwilliger MM, Berger PA, Hollister LE and Csernansky JG, A comparison of the neurochemical and behavioral effects of clenbuterol and desipramine. *Eur J Pharmacol* **134**: 131–136, 1987.
14. Edwards DJ, Sorisio D, Knopf S and Mujumdar S, Assay for L-*p*-tyrosine in plasma and brain by column liquid chromatography with electrochemical detection using *m*-tyrosine as the interanl standard. *J Chromatogr* (Biomed Applic) **383**: 142–147, 1982.
15. Ambrose JA, A shortened method for the fluorometric determination of phenylalanine. *Clin Chem* **15**: 15–23, 1969.
16. Schwenk WF, Berg PJ, Beaufrere B, Miles JM and Haymond MW, Use of *t*-butyldimethylsilylation in the gas chromatographic/mass spectrometric analysis of physiologic compounds found in plasma using electron-impact ionization. *Anal Biochem* **141**: 101–109, 1984.

17. Hallberg H, Almgren O and Svensson TH, Increased brain serotonergic and noradrenergic activity after repeated systemic administration of the beta-2 adrenoceptor agonist salbutamol, a putative antidepressant drug. *Psychopharmacology* **73**: 201–204, 1981.
18. Waldmeier PC, Stimulation of central serotonin turnover by β-adrenoceptor agonists. *Naunyn-Schmiedebergs Arch Pharmacol* **317**: 115–119, 1981.
19. Middlemiss DN, Blockade of the central 5-HT autoreceptor by β-adrenoceptor antagonists. *Eur J Pharmacol* **120**: 51–56, 1986.
20. Middlemiss DN, Błakeborough L and Leather SR, Direct evidence for an interaction of β-adrenergic blockers with the 5-HT receptor. *Nature* **267**: 289–290, 1977.
21. Peroutka SJ, Serotonin receptors. In: *Psychopharmacology: The Third Generation of Progress* (Ed. Meltzer HY), pp. 303–311. Raven Press, New York, 1987.
22. Fernstrom JD and Faller DV, Neutral amino acids in the brain: changes in response to food ingestion. *J Neurochem* **30**: 1531–1538, 1978.
23. McElroy JF and O'Donnell JM, Discriminative stimulus properties of clenbuterol: evidence for *beta* adrenergic involvement. *J Pharmacol Exp Ther* **245**: 155–163, 1988.
24. Hutson PH, Knott PJ and Curzon G, Effect of isoprenaline infusion on the distribution of tryptophan, tyrosine and isoleucine between brain and other tissues. *Biochem Pharmacol* **29**: 509–516, 1980.
25. Woods PB and Robinson ML, An investigation of the comparative liposolubilities of β-adrenoceptor blocking agents. *J Pharm Pharmacol* **33**: 172–173, 1981.
26. Eriksson T and Carlsson A, β-Adrenergic control of brain uptake of large neutral amino acids. *Life Sci* **42**: 1583–1589, 1988.
27. Beer M, Richardson A, Poat J, Iversen LL and Stahl SM, *In vitro* selectivity of agonists and antagonists for $beta_1$- and $beta_2$-adrenoceptor subtypes in rat brain. *Biochem Pharmacol* **37**: 1145–1151, 1988.
28. Ordway GA, O'Donnell JM and Frazer A, Effects of clenbuterol on central *beta*-1 and *beta*-2 adrenergic receptors of the rat. *J Pharmacol Exp Ther* **241**: 187–195, 1987.
29. Badawy AA-B and Evans M, Inhibition of rat liver tryptophan pyrrolase activity and elevation of brain tryptophan concentration by acute administration of small doses of antidepressants. *Br J Pharmacol* **77**; 59–67, 1982.
30. Frances H, Renwart N, Danti S, Cash R, Raisman R and Simon P, Beta-adrenergic agonists reduce spontaneous motor activity through either β1 or β2 receptors. *Pharmacol Biochem Behav* **26**: 11–15, 1987.