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COMMUNICATIONS

A study of the effects of chronic salbutamol on rat brain monoaminergic systems

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In addition to its bronchodilator action, the selective β_2-adrenoceptor agonist salbutamol has been reported to be an efficacious antidepressant (Lecrubier et al 1980). Furthermore, acute studies have demonstrated that it possesses pharmacological actions common to tricyclic antidepressants. For example, the drug antagonizes reserpine-induced hypothermia in mice (Francès et al 1978; Souto et al 1979; Przegalinski et al 1980; Ross 1980) and, like 5-hydroxytryptamine (5-HT) uptake inhibitors, potentiates the behavioural syndrome elicited by L-5-hydroxytryptophan (5-HTP) (Ortmann et al 1981). These actions appear to be central in origin since they are elicited by the intraventricular administration of the drug (Francès et al 1979; Ortmann et al 1981). In addition, the ability of systemically administered salbutamol to potentiate L-5-HTP is attenuated by propranolol and not by practolol (Ortmann et al 1981). The former β-adrenoceptor antagonist readily penetrates the brain whereas the latter does not. The above observations can be interpreted as indicating that salbutamol, like classical tricyclic antidepressants, interacts with central monoaminergic systems. The monoamines noradrenaline (NA), dopamine (DA) and 5-HT are considered to play a critical role in the mechanisms of action of antidepressant drugs (for review, cf. Sugrue 1981a) and the interaction of salbutamol with central monoaminergic systems may account for the postulated antidepressant activity of the drug.

Acute studies are of limited value in attempting to understand the mechanisms of action of antidepressants since there is a lag phase of one to three weeks before both tricyclic and atypical antidepressants elicit a beneficial effect. Antidepressants evoke a number of adaptive changes following their chronic administration (cf. Sugrue 1981a, b) and such changes may be relevant to their modes of action. Examples of drug-induced adaptive changes include modifications in rat brain monoamine turnover, the induction of subsensitive presynaptic α_2-adrenoceptors and a reduction in the number of central β-adrenoceptor binding sites. The aim of this study was to determine if chronically-administered salbutamol elicited similar adaptive changes.

Methods

Male Sprague-Dawley rats, 150–180 g at the commencement of drug treatment were used. Rats were in good health during the administration schedule and no fatalities occurred. Salbutamol (generously supplied by Glaxo) was dissolved in distilled water and 5 mg kg^{-1}, dose as base, was injected i.p. every 12 h (8·00 and 20·00) for 14 days. Controls received distilled water. Unless stated otherwise, rats were killed by stunning followed by decapitation 12 h after the last injection of salbutamol. In the clonidine interaction studies, the drug (25 μg kg^{-1}, i.p.) or its vehicle (0·9% NaCl) was injected 12 h after the last dose of salbutamol and rats were killed 3 h later. In the acute experiment, salbutamol (5 mg kg^{-1}, i.p.) was injected 30 min before clonidine or its vehicle and rats were killed 3 h later. After death, tissues were immediately frozen in liquid nitrogen and then stored at −80 °C. Tissue levels of NA, DA, 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylethyleneglycol sulphate (MHPG-SO_4) were assayed spectrophotofluorometrically, as described by Sugrue (1980). Results are expressed as ng g^{-1} wet tissue and are corrected to 100% recovery based on concurrently run internal standards. Potential changes in brain NA and DA turnover were assessed by determining the effect of drug pretreatment on the decline in brain NA and DA content elicited by the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine (AMPT). Twelve h after the last injection of salbutamol, AMPT (200 mg kg^{-1}) was injected i.p. and rats killed 3 h later. 5-HT turnover was quantified by means of the probenecid-induced increase in brain 5-HIAA content. Again, 12 h after cessation of drug treatment, probenecid (200 mg kg^{-1}) was injected i.p. and rats killed 1 h later. In the in vivo experiments, [^{3}H]clonidine and [^{3}H]dihydroalprenolol (DHA) binding was assayed in parallel using the same frontal cortex tissue. Tissue was homogenized in 20 volumes of 50 mM Tris-HCl buffer, pH 7·7 at 25 °C, and the homogenate divided into two aliquots. Following centrifugation, pellets were resuspended in appropriate buffers and assayed for [^{3}H]clonidine (U'Prichard et al 1979) and [^{3}H]DHA (Bylund & Snyder 1976) binding using conventional techniques. [4-^{3}H]clonidine hydrochloride (23·8 Ci $mmol^{-1}$) and (−)-propyl-[1,2,3-^{3}H]-dihydroalprenolol hydrochloride (44·9 Ci $mmol^{-1}$) were purchased from New England Nuclear. Each result is the mean of at least five determinations and statistical significance was determined using Student's *t*-test (two-tailed).

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Results

Chronically administered salbutamol was devoid of effect on rat brain steady state levels of NA, DA, DOPAC and 5-HT (Table 1). However, levels of 5-HIAA, the major metabolite of 5-HT in rat brain, were significantly elevated. This observation suggests a drug-induced increase in central 5-HT turnover. In control rats the difference in brain pre- and post-probenecid 5-HIAA levels was 103 ± 9 ng g^{-1} (n = 6). This difference was significantly increased to 138 ± 3 ng g^{-1} (n = 6) ($P < 0.01$) in the brains of treated rats. Thus, long-term salbutamol is associated with an increase in rat brain 5-HT turnover. Chronically administered salbutamol had no effect on rat brain steady state levels of DOPAC (Table 1) and MHPG-SO_4 (Table 3) thus suggesting that DA and NA turnover were unaltered. This was confirmed by the inability of the drug to change the AMPT-induced reduction in rat brain NA and DA levels (Table 2). The ability of clonidine (25 μg kg^{-1} i.p.) to lower rat brain MHPG-SO_4 levels was unaltered by acutely and chronically administered salbutamol (Table 3).

Scatchard analysis of [^{3}H]clonidine and [^{3}H]DHA binding to rat frontal cortex membranes, using six concentrations of ligand ranging from 0·2 to 10 nM, revealed the presence of a single binding component for each radioligand. The Kd and Bmax values for [^{3}H]clonidine were 1·4 nM and 4·5 pmol g^{-1}. Corresponding values for [^{3}H]DHA binding were 2·0 nM and 11·1 pmol g^{-1} respectively (Sugrue, submitted for publication). In the presence of a ligand concentration of 0·5 nM, the K_i values of salbutamol for cortical [^{3}H]clonidine and [^{3}H]DHA binding sites were 0·1 mM and 3·2 μM respectively. Hence, the affinity of the drug for both recognition sites is extremely weak. Rat frontal cortex binding of [^{3}H]clonidine and [^{3}H]DHA in the presence of a ligand concentration of 0·5 n mol was unaltered by chronically administered salbutamol (controls 0·79 ± 0·04, 1·85 ± 0·09; treated 0·83 ± 0·05, 1·94 ± 0·17 p mol g^{-1} tissue respectively n = 6).

Table 1. Effect of twice daily administration of salbutamol (5 mg kg^{-1} i.p.) for 14 days on rat brain steady state levels of NA, DA, DOPAC, 5-HT and 5-HIAA. Rats were killed 12 h after the last injection. Each result is the mean ± s.e.m. of at least five determinations.

	Steady state levels (ng g^{-1})				
	NA	DA	DOPAC	5-HT	5-HIAA
Control	407 ± 8	1085 ± 37	111 ± 3	390 ± 33	315 ± 25
Treated	423 ± 8	1087 ± 52	109 ± 2	366 ± 48	382 ± 16*

* Differs from control, $P < 0.05$.

Table 2. Effect of twice daily administration of salbutamol (5 mg kg^{-1}, i.p.) for 14 days on AMPT-induced fall in rat brain content of NA and DA. AMPT (200 mg kg^{-1}) was injected i.p. 12 h after last drug injection and rats were killed 3 h later. Each result is the mean ± s.e.m. of six determinations and is expressed as percent of appropriate steady state level at time of AMPT injection.

	% of steady state	
	NA	DA
Control	60·0 ± 1·6	38.2 ± 1·8
Treated	63·9 ± 3·0	43·4 ± 2·2

Table 3. Effect of acute and chronic administration of salbutamol on clonidine-induced decrease in rat brain MHPG-SO_4 content. Acutely administered salbutamol (5 mg kg^{-1}) was injected i.p. 30 min before clonidine (25 μg kg^{-1} i.p.) or its vehicle (0·9% NaCl) and rats were killed 3 h later. In chronic studies, salbutamol (5 mg kg^{-1}) was injected i.p. twice daily for 14 days and 12 h after the last injection, clonidine or its vehicle was injected i.p. and rats were killed 3 h later. Each result is the mean ± s.e.m. of 6–8 determinations.

	Brain MHPG-SO_4 levels (ng g^{-1})			
	Control	Clonidine	Salbutamol	Salbutamol + clonidine
Acute	115 ± 6	85 ± 2*	123 ± 3	98 ± 9†
Chronic	128 ± 9	94 ± 4*	113 ± 4	83 ± 5‡

* Differs from control, $P < 0.01$.
† Differs from drug-treated, $P < 0.05$.
‡ Differs from drug-treated, $P < 0.01$.

Discussion

The above observations reveal that chronic salbutamol is associated with an increased turnover of 5-HT in rat brain. Moreover, acutely administered salbutamol increases 5-HT turnover as indicated by the observation that 30 min pretreatment of 5 mg kg^{-1} significantly ($P < 0.01$) increased the probenecid-induced augmentation of 5-HIAA levels by 33·2 ± 9·7% (n = 5) (unpublished data). Others have also observed an increase in rat brain 5-HT turnover after acute (Waldmeier 1981) and chronic (Hallberg et al 1981) salbutamol. In this respect, salbutamol differs from 5-HT uptake inhibitors since the latter, on acute administration, decrease the turnover of the monoamine (Carlsson & Lindqvist 1978). Furthermore, salbutamol is essentially devoid of effect on central 5-HT re-uptake as demonstrated by the finding that the ability of rat hypothalmic synaptosomes to take up [^{3}H]-5-HT (26 nM) was inhibited 25·1 ± 2·0% (n = 4) by 10^{-5} M salbutamol (unpublished data). In addition to its lack of effect on 5-HT re-uptake, salbutamol would appear to be devoid of effect on central 5-HT receptors in vivo as indicated by its ineffectiveness in tests considered to be predictive for central 5-HT receptor agonists and antagonists (Przegalinski et al 1980). Further evidence against a direct action of salbutamol on central post-synaptic 5-HT receptors is the observation that the ability of the β-adrenoceptor agonist to potentiate the L-5-HTP behavioural response is attenuated by the prior destruction of 5-HT nerve terminals by the neurotoxin 5,7-dihydroxytryptamine (Ortmann et al

1981). The salbutamol-induced increase in 5-HT turnover could be due to the drug either directly releasing the monoamine from its nerve terminals or to a blockade of presynaptic 5-HT receptors. However, evidence against these possibilities is the fact that pretreatment with propranolol prevents both the salbutamol-induced increase in 5-HT turnover (Waldmeier 1981) and the ability of the drug to potentiate the L-5-HTP behavioural response (Ortmann et al 1981). These findings point to the effects of salbutamol on 5-HT turnover being mediated by β-adrenoceptors. The firing activity of 5-HT cells of the rat dorsal raphe nucleus is dependent on a tonically active, central adrenergic system. However, this system would appear to have the characteristics of an α_1-, and not β-, adrenoceptor (Baraban & Aghajanian 1980). At this point in time, the precise mechanism whereby acute and chronic salbutamol increases rat brain 5-HT turnover is far from clear.

In contrast to its ability to increase 5-HT turnover, chronic salbutamol fails to alter rat brain NA and DA turnover, as assessed by the AMMT model and by changes in MHPG-SO_4 and DOPAC levels. However, an increase in central NA, but not DA, turnover has been reported by Hallberg et al (1981). Certain chronic forms of antidepressant therapy, such as desipramine (McMillen et al 1980; Sugrue 1981c) and electroconvulsive shock (Heal et al 1981), induce subsensitive presynaptic α_2-adrenoceptors, as assessed by their effect on the ability of clonidine to lower rat brain MHPG-SO_4 levels. The ineffectiveness of chronic salbutamol to alter the reduction in rat brain MHPG-SO_4 content elicited by clonidine (25 μg kg^{-1} i.p.) clearly indicates that the drug is not associated with the induction of subsensitive presynaptic α_2-adrenoceptors. Also there is the inability of chronic salbutamol to alter rat frontal cortex [^{3}H]clonidine binding. However, to assume that alterations in central [^{3}H]clonidine binding sites reflect alterations in the functioning of central presynaptic α_2-adrenoceptors is open to question since drugs such as desipramine and mianserin, which on chronic administration alter the sensitivity of rat brain α_2-adrenoceptors, fail in parallel experiments to change [^{3}H]clonidine binding (Sugrue 1982).

An effect common to many forms of long-term antidepressant therapy is a reduction in the number of central β-adrenoceptors (Reisine 1981). Often, this is associated with a diminished sensitivity of NA-stimulated adenylate cyclase activity in rat brain slices and the down-regulation in β-adrenoceptor responsiveness is currently postulated to play a critical role in the mechanism of action of antidepressants (Mobley & Sulser 1981). However, the two phenomena are not necessarily coupled since chronic mianserin and zimelidine induce a subsensitive NA-stimulated adenylate cyclase system yet fail to alter β-adrenoceptor binding (Mobley & Sulser 1981). The results of the present study indicate that chronic salbutamol fails to alter rat frontal cortex [^{3}H]DHA binding and thus confirms the observation of others (Hall et al 1980). In this respect, salbutamol differs from many, but not all forms, of long-term antidepressant therapy. The effect of chronic salbutamol on a system of central β-adrenoceptor functionality such as the adenylate cyclase system has yet to be reported although it has recently been observed that the salbutamol-induced elevation in human plasma cAMP levels decreases with chronic administration of the drug and exhibits a time course paralleling the antidepressant effect of the drug. However, it would be premature to assume that an analogous induced subsensitivity occurs centrally since vascular β-adrenoceptors in muscle tissue constitute the major source of the adrenoceptor agonist-induced rise in plasma cAMP (Lerer et al 1981).

In summary, the chronic administration of salbutamol to rats does not share with certain more traditional forms of antidepressant therapy the ability to down-regulate the number of central β-adrenoceptors or to induce subsensitive presynaptic α_2-adrenoceptors. Long-term salbutamol is associated with increased turnover of 5-HT in rat brain by a yet to be elucidated mechanism of action.

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Effect of norfenfluramine and two structural analogues on brain 5-hydroxyindoles and serum prolactin in rats

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Fenfluramine and norfenfluramine elevate serum prolactin in rats (Fuller et al 1976), apparently by a mechanism involving the release of 5-hydroxytryptamine (Quattrone et al 1978). Here we describe studies with two structural analogues of norfenfluramine, one of which depletes 5-hydroxytryptamine as do norfenfluramine and fenfluramine, the other of which does not. The effects of these analogues on serum prolactin are consistent with the idea that the elevation of prolactin is mediated by 5-hydroxytryptamine release.

Male Wistar rats, 190–210 g, obtained from Harlan Industries, Cumberland, Indiana, had free access to food and water. The compounds were synthesized in the Lilly Research Laboratories as hydrochloride salts and were injected i.p. in aqueous solutions (0·05 mmol kg^{-1}). Rats were decapitated 1 or 6 h after injection. Whole brains were rapidly excised, frozen on dry ice, and stored frozen before analysis. 5-Hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations were determined spectrofluorometrically after condensation with *o*-phthalaldehyde (Miller et al 1970). Blood collected from the cervical wound was allowed to clot, then serum obtained after centrifugation was stored frozen. Prolactin concentration in serum was measured by radioimmunoassay using the NIAMDD kit and is expressed as ng of NIAMDD rat prolactin RP-1 ml^{-1}.

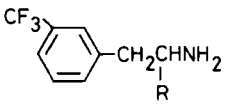

Table 1 shows the effect of norfenfluramine (α-methyl-m-trifluoromethyl-phenylethylamine) on 5-HT and 5-HIAA concentrations in brain and on prolactin concentrations in the serum of male rats. Norfenfluramine was injected at 12 mg kg^{-1}, and the analogues were injected at equimolar doses (0·05 mmol kg^{-1}). Norfenfluramine depleted 5-HT and 5-HIAA in brain and elevated serum prolactin, effects that have been reported earlier (Fuller et al 1976, 1978). The α-ethyl analogue of norfenfluramine likewise depleted the 5-hydroxyindoles and elevated serum prolactin. In contrast, the α-n-propyl analogue had no effect either on brain 5-hydroxyindoles or on serum prolactin.

Fenfluramine is thought to cause a rapid release of 5-HT (Trulson & Jacobs 1976; Kannengiesser et al 1976; Clineschmidt & McGuffin 1978), and this release of 5-HT not only may produce the elevation of serum prolactin observed with fenfluramine and norfenfluramine but also results in depletion of 5-hydroxyindole levels. Our data showing that the α-ethyl analogue of norfenfluramine depletes 5-hydroxyindoles and elevates serum prolactin, whereas the α-propyl analogue, which does not deplete 5-hydroxyindoles, does not elevate serum prolactin, are compatible with the idea (Quattrone et al 1978) that the release of 5-HT mediates the increase in serum prolactin.

The compounds used were racemates, and the stereoisomers of norfenfluramine and fenfluramine have been shown to differ in their potency in affecting brain 5-hydroxytryptaminergic and dopaminergic systems (Crunelli et al 1980; Jori et al 1973; Bendotti et al 1980). For instance, the (−)-isomer of fenfluramine has been suggested to influence dopamine neurons directly, whereas the

Table 1. Structure-activity relationships in the lowering of brain 5-hydroxyindoles and elevation of serum prolactin in rats by alkyl-substituted m-trifluoromethyl-phenylethylamines. All compounds were injected i.p. at 0·05 mmol kg^{-1}. Brain concentrations of 5-hydroxytryptamine (5-HT) and of 5-hydroxyindoleacetic acid (5-HIAA) were measured at 6 h, serum prolactin at 1 h after injection. Mean values ± standard errors for 5 rats per group are shown.

	Brain 5-hydroxyindoles, μg g^{-1}		Serum prolactin
	5-HT	5-HIAA	ng ml^{-1}
Vehicle-treated control	0·57 ± 0·02	0·57 ± 0·02	8·1 ± 1·8
R = Methyl (norfenfluramine)	0·22 ± 0·02*	0·33 ± 0·04*	74·2 = 11·2*
R = Ethyl	0·40 ± 0·01*	0·37 ± 0·02*	85·9 ± 6·0*
R = n-Propyl	0·57 ± 0·01	0·53 ± 0·02	9·3 ± 3·0

* Significant difference from control group ($P < 0·01$).

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