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Effect of butaclamol, a new neuroleptic, on serotoninergic mechanisms

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Butaclamol (1·0–0·1 mg kg^{-1}, i.p.) and spiroperidol (1·0–0·5 mg kg^{-1}, i.p.), but not (−)-butaclamol (15 mg kg^{-1}, i.p.), blocked the hyperactivity induced in rats by tranylcypromine-L-tryptophan pretreatment. Neither butaclamol nor spiroperidol altered the accumulation of brain 5-HT following pargyline or the decline of brain 5-HT following inhibition with the tryptophan hydroxylase inhibitor α-propyldopacetamide thus indicating that butaclamol and spiroperidol do not affect either the synthesis or the turnover of brain 5-HT. It is concluded that the antagonism of the tranylcypromine-L-tryptophan-induced hyperactivity by butaclamol and spiroperidol is due to their blockade of dopaminergic receptors rather than an action on neuronal serotoninergic mechanisms.

Butaclamol is a new neuroleptic (Voith & Herr, 1975) and antipsychotic (Mielke, Gallant & others, 1975) drug which has been shown to accelerate *in vivo* the turnover of dopamine, and in higher doses noradrenaline, in whole brain (Lippmann & Pugsley, 1975) and dopamine in striatum (Lippmann, Pugsley & Merker, 1975). *In vitro*, the drug also inhibits the dopamine-stimulated increase of olfactory tubercle (Lippmann & others, 1975) and striatal (Miller, Horn & Iversen, 1975) adenyl cyclase. Further studies have indicated that, *in vivo*, butaclamol increases the affinity of tyrosine hydroxylase for the pteridine cofactor in striatum and nucleus accumbens (Zivkovic, Guidotti & Costa, 1975) and, *in vitro*, inhibits the electrically-stimulated release of [^{3}H]dopamine from rat striatal slices (Seeman & Lee, 1975).

The new neuroleptic agent methiothepin in addition to blocking noradrenaline and dopamine receptors (Keller, Bartholini & Pletscher, 1973; Lloyd & Bartholini, 1974) also induces pontogeniculate-occipital spikes in cats (Monachon, Burkard & others, 1972) and elevates brain 5-hydroxyindoles in animals (Monachon & others, 1972; Lloyd & Bartholini, 1974; Jacoby, Shabshelowitz & others, 1975). On the basis of their findings it has been suggested that methiothepin increases 5-hydroxytryptamine (5-HT, serotonin) turnover by blocking central (5-HT) receptors.

Some pharmacological evidence (Jacobs, 1974) based upon a 5-HT-mediated behaviour syndrome in rats has indicated that spiroperidol, a relatively specific dopamine receptor blocking neuroleptic agent (Andén, Butcher & others, 1970), may also block 5-HT receptors. If so, an increase in 5-HT turnover could be expected.

The purpose of the present study was two-fold: to examine the effect of butaclamol on brain 5-HT turnover and on 5-HT-mediated behaviour as judged in the tranylcypromine-L-tryptophan-induced hyperactivity syndrome in rats (Grahame-Smith, 1971a, b); to determine whether the suggested 5-HT receptor blocking activity of spiroperidol (Jacobs, 1974) is manifested as an alteration in 5-HT turnover.

METHODS AND MATERIALS

Male Sprague-Dawley rats (150–160 g; Canadian Breeding Laboratories) were used. All drugs were administered intraperitoneally.

Effect on the hyperactivity following tranylcypromine and L-tryptophan

The method of Jacobs (1974) was followed except tranylcypromine was used instead of pargyline as the monoamine oxidase inhibitor. Both of these drugs when combined with L-tryptophan cause a characteristic behavioural syndrome which includes hyperactivity (Grahame-Smith, 1971a, b). The test drug was given 30 min after the L-tryptophan, the tranylcypromine 30 min before the L-tryptophan. The hyperactivity was judged on an all or none basis 60 min after the test drug administration; it consisted of continuous locomotor movements characterized by compulsive jerky circling movements produced by a reciprocal treading of the forepaws with the hindpart held relatively stationary.

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Table 1. *Behavioural effect of butaclamol and spiroperidol in rats pretreated with tranylcypromine and tryptophan.*

Treatment	Dose (mg kg^{-1}, i.p.)	No. rats with hyperactivity syndrome[a]
Saline	—	10/10
Butaclamol	1·0	0/10
	0·5	1/9
	0·1	6/10
(—)-Butaclamol	15·0	5/5
Saline	—	8/10
Spiroperidol	1·0	1/9
	0·75	1/10
	0·5	2/5

[a] As described in methods. The tranylcypromine (20 mg kg^{-1}, i.p.) was given 30 min before the L-tryptophan (100 mg kg^{-1}, i.p.) and the test drugs 30 min after the L-tryptophan. Ratios indicate the number of rats displaying hyperactivity over the total number of animals in the test at 60 min after the test drug administration.

Effect on brain 5-HT synthesis and turnover

In the determination of the effect of the test drugs alone and together with the tranylcypromine-L-tryptophan treatment on brain 5-HT and tryptophan, the conditions described above for the behavioural syndrome were employed. In other studies the effect of the test drugs on the accumulation of brain 5-HT when given 1 h before the tranylcypromine-L-tryptophan treatment was also determined.

Changes in brain 5-HT turnover by the test drugs were also estimated by studying their effect on the decline of 5-HT stores following treatment with supramaximal doses of the rapid acting tryptophan hydroxylase inhibitor α-propyldopacetamide (H22/54; Carlsson, Corrodi & Waldeck, 1963; Andén, Corrodi & Fuxe, 1969). The test drug was injected 15 min before the administration of H22/54 (500 mg kg^{-1}) and the animals killed 3 h after the latter treatment.

In the above studies the animals were decapitated, the brains quickly removed, weighed and frozen at −20° overnight. Brain 5-HT content was analysed by the method of Curzon & Green (1970). Brain tryptophan was determined by the method of Denckla & Dewey (1967) as modified by Bloxam & Warren (1974).

The following drugs were used: (±)-butaclamol hydrochloride and (−)-butaclamol hydrochloride (Ayerst Laboratories; Bruderlein, Humber & Voith, 1975; Humber, Bruderlein & Voith, 1975); spiroperidol (Janssen Pharmaceuticals), tranylcypromine sulphate (Parnate; Smith, Kline & French Laboratories); the latter drugs were gifts from the respective companies. L-Tryptophan was obtained from Calibiochem and α-propyldopacetamide (H22/54) from Aldrich Laboratories.

RESULTS

Effect on the tranylcypromine-L-tryptophan-induced behavioural syndrome

In rats pretreated with tranylcypromine and L-tryptophan, hyperactivity was produced (Table 1). At the doses examined, butaclamol (1·0–0·1 mg kg^{-1}) and spiroperidol (1·0–0·5 mg kg^{-1}), administered 30 min after L-tryptophan, antagonized this hyperactivity generally in a dose-related fashion. (−)-Butaclamol was ineffective at a dose level 15 times that of the highest dose of butaclamol.

Effect on brain 5-HT synthesis and turnover

Butaclamol and spiroperidol administered 30 min after the L-tryptophan neither altered the accumulation of 5-HT after tranylcypromine and L-tryptophan nor the steady state level of 5-HT when given alone (Table 2). Butaclamol caused an elevation of brain tryptophan; spiroperidol had no effect. When

Table 2. *Effect on brain 5-HT and tryptophan concentrations and on the increased accumulation produced by tranylcypromine and L-tryptophan in rats.*

Treatment	Brain tryptophan (μg g^{-1} ± s.e.)	Brain 5-HT (μg g^{-1} ± s.e.)
Saline	4·59 ± 0·35	0·39 ± 0·01
Butaclamol	5·73 ± 0·20*	0·37 ± 0·02
Spiroperidol	4·86 ± 0·26	0·36 ± 0·02
Tranylcypromine + L-tryptophan + saline	36·59 ± 2·52	0·92 ± 0·05
Tranylcypromine + L-tryptophan + butaclamol	45·84 ± 2·63*	0·99 ± 0·03
Tranylcypromine + L-tryptophan + spiroperidol	36·47 ± 2·78	0·97 ± 0·03

Groups of 5 rats were employed. Three groups received saline, butaclamol (1 mg kg^{-1}, i.p.) or spiroperidol (1 mg kg^{-1}, i.p.) and were killed 60 min later. Three groups received tranylcypromine (20 mg kg^{-1}, i.p.) followed 30 min by L-tryptophan (100 mg kg^{-1}, i.p.) and saline or test drug as indicated 30 min after the L-tryptophan; the animals were killed 60 min after the test drug injection.

*$P < 0·01$ compared with respective saline controls.

these drugs were each administered 1 h before the tranylcypromine-L-tryptophan, and the animals (5 per group) killed 1 h after the L-tryptophan administration, no alteration in the 5-HT accumulation was observed after these test drugs; the concentration of 5-HT (μg g^{-1} $\pm$ s.e.) was 0·81 $\pm$ 0·06, 0·71 $\pm$ 0·03 and 0·78 $\pm$ 0·04 after saline, butaclamol and spiroperidol, respectively.

Butaclamol and spiroperidol alone did not alter brain 5-HT nor did they affect significantly the H22/54-induced depletion of brain 5-HT indicating a lack of an effect on brain 5-HT turnover (Table 3).

Table 3. *Effect on brain 5-HT concentrations and on the H22/54-induced depletion of 5-HT in rat.*

Treatment	Dose (mg kg^{-1}, i.p.)	5-HT (μg g^{-1} $\pm$ s.e.)
Saline	—	0·33 $\pm$ 0·01[a]
Butaclamol	1	0·36 $\pm$ 0·01[b]
Spiroperidol	1	0·35 $\pm$ 0·02[c]
H22/54	500	0·22 $\pm$ 0·03[d]
Butaclamol + H22/54	1 + 500	0·26 $\pm$ 0·03[e]
Spiroperidol + H22/54	1 + 500	0·26 $\pm$ 0·02[f]

Drugs were injected 15 min before the α-propyldopacetamide (H22/54). Animals were killed 3 h after the H22/54. There were 10 rats in saline group and 5 in each of the treatment groups.
[a,d] $P < 0·01$, [a,b;a,c;d,e;d,f] N.S.

DISCUSSION

In the present studies a single injection of the neuroleptics butaclamol and spiroperidol blocked the hyperactivity syndrome resulting from administration of the monoamine oxidase inhibitor tranylcypromine and L-tryptophan. Such activity has been reported for the neuroleptics chlorpromazine (Grahame-Smith, 1971b) and spiroperidol (Jacobs, 1974). It has been suggested that the antagonism of such hyperactivity by spiroperidol may be due to 5-HT receptor blockade (Jacobs, 1974). On this basis it might be expected that spiroperidol would alter the synthesis and/or turnover of brain 5-HT. The present findings indicate that in rats treated with a monoamine oxidase inhibitor and L-tryptophan, butaclamol and spiroperidol did not affect the resulting increased synthesis of 5-HT. Chlorpromazine also did not affect 5-HT synthesis (Grahame-Smith, 1971b). Butaclamol and spiroperidol did not affect the H22/54-induced fall in brain 5-HT, findings which represent further evidence against an effect on 5-HT turnover. These findings that butaclamol and spiroperidol do not affect 5-HT synthesis or turnover indicate that neither a blockade of the 5-HT receptors nor an interference with the presynaptic synthesis or release of 5-HT are likely to account for their inhibitory action of the hyperactivity syndrome.

A number of psychotropic drugs that increase brain tryptophan also increase the synthesis of brain 5-HT (Tagliamonte, Tagliamonte & others, 1971). Butaclamol increased brain tryptophan, but did not affect brain 5-HT synthesis, under the conditions of the present study. Other investigators (Hamon & Glowinski, 1974; Morot-Gaudry, Hamon & others, 1974) have also concluded that the synthesis and release of 5-HT is not always dependent on fluctuations of tryptophan metabolism. The present findings with regard to butaclamol add further support to the above conclusion, i.e., that 5-HT synthesis and release are not always related to alterations in tryptophan metabolism.

Initial reported studies had indicated that the hyperactivity syndrome appeared to be due to an increased synthesis and accumulation of 5-HT with a subsequent spillover of the 5-HT, and/or 5-HT derivatives formed from the free 5-HT pool, onto postsynaptic 5-HT receptors (Grahame-Smith, 1971a,b). As this hyperactivity syndrome was blocked by depletion of brain dopamine, but not brain noradrenaline, it was postulated that the hyperactivity syndrome is dependent upon both normally functioning dopaminergic and serotoninergic neuronal systems for its full expression (Green & Grahame-Smith, 1974). More recently, no correlation between the hyperactivity syndrome and the rate of accumulation of brain 5-HT was found (Foldes & Costa, 1975). The latter investigators postulated that an indole metabolite other than 5-HT, such as tryptamine, could trigger the hyperactivity by acting on catecholaminergic neurons, probably dopaminergic, thus causing the hyperactivity that would be catecholamine, and not indoleamine, mediated. The present studies indicate that butaclamol and spiroperidol inhibit the hyperactivity syndrome but do not alter either 5-HT synthesis or turnover, the latter findings being unexpected if the drugs were affecting neuronal serotoninergic mechanisms. Both butaclamol (see introduction for references) and spiroperidol (Andén & others, 1970) block specifically dopamine receptors in the doses used in this study. Thus, it would appear that butaclamol and spiroperidol most probably inhibit the hyperactivity syndrome by blocking brain dopamine receptors. This is supported by the findings that α-methyl-*p*-tyrosine (Green &

Grahame-Smith, 1974; Foldes & Costa, 1975) and 6-hydroxydopamine (Foldes & Costa, 1975), drugs which interfere with catecholamine systems, also block the hyperactivity syndrome. Additional evidence is the present observation that (−)-butaclamol is ineffective in inhibiting the hyperactivity syndrome. Previous pharmacological (Humber & others, 1975) and biochemical (Lippmann & others, 1975; Miller & others, 1975; Zivkovic & others, 1975) findings have shown that (−)-butaclamol is ineffective as a blocker of the dopaminergic receptor. Based upon the present studies, the possibility that a blockade of 5-HT receptors and/or interference with 5-HT synthesis or release accounts for their effect does not appear likely under the conditions used.

Thus, the present findings indicate that the new neuroleptic butaclamol, like spiroperidol, does not affect brain 5-HT synthesis or turnover. The inhibitory action of these drugs on the monoamine oxidase inhibitor-L-tryptophan-induced hyperactivity syndrome appears to be due to their known blockade of dopamine receptors rather than an action on neuronal serotoninergic mechanisms.

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