J. Pharm. Pharmacol. 1982, 34: 314–317
Received October 8, 1981

0022–3573/82/050314–04 $02.50/0

Neurochemical effects of buspirone, a novel psychotropic drug, on the central cholinergic system

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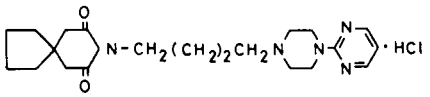
Buspirone, a novel psychotropic anxioselective agent, produced a dose-dependent decrease in the level of acetylcholine in the striatum of the rat. The maximum effect of about 25–30% was produced at the dose of 20 mg kg^{-1}. A smaller decrease of 10% was also found in the n. accumbens-olfactory tubercle while other brain regions were unaffected. The drug did not alter striatal choline acetyltransferase or acetylcholinesterase activities and was feeble in displacing [^{3}H]dexetimide from its specific muscarinic binding sites. The effect of buspirone in lowering acetylcholine content was more marked and longer lasting in the striatum of female than male rats. Buspirone proved to be weak as a blocker of the dopamine receptor agonist, apomorphine, and it appears that only a small proportion of the decrease in striatal acetylcholine content can be attributed to the blockade of dopamine receptors. Rapid homologous tolerance to an acute challenge with buspirone on striatal acetylcholine was achieved within seven days of its chronic administration, and, unlike clozapine, a cross tolerance of buspirone to chronic haloperidol treatment was also observed. Other data indicating that the drug differed from haloperidol both qualitatively and quantitatively on dopaminergic neurochemical parameters, and the fact that it is not cataleptogenic, suggest that buspirone cannot be considered a typical neuroleptic agent. The possibility that buspirone may act as an agonist at certain presynaptic dopamine receptors, which could translate into a fall in striatal acetylcholine content, is discussed.

Buspirone (MJ 9022), 8-(4-(4-(2-pyrimidinyl)-piperazinyl)butyl)-8-azaspiro(4,5) decane-7,9-dione hydrochloride is a novel psychotropic drug with a pharmacological profile different from other known classes of drugs. It inhibits amphetamine lethality in grouped mice and blocks apomorphine induced emesis, but unlike neuroleptic agents, buspirone is inactive as a cataleptogenic agent (Allen et al 1974). Behavioural studies in animals as well as clinical



Buspirone hydrochloride

observations have revealed that buspirone possesses an anxioselective pharmacological profile. Specifically, while buspirone shares diazepam's anti-anxiety and anti-aggressive properties, it does not elicit sedative-hypnotic or anticonvulsant effects (Allen et al 1974; Goldberg & Finnerty 1979; Tompkins et al 1980).

A major neurochemical effect of buspirone is on the dopaminergic system. Relatively low doses of the drug (2·5–10 mg kg^{-1}), induce a dose-dependent increase in striatal dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), the acid metabolites of dopamine (S. Algeri, manuscript in preparation). In view of the link existing in the striatum between inhibitory nigro-neostriatal dopaminergic fibres and the cholinergic neurons intrinsic to this area (Ladinsky & Consolo 1979; Consolo et al 1974) and considering the fact that several psychotropic agents can affect the central cholinergic system (Consolo et al 1972) it was decided to investigate the effect of buspirone on brain cholinergic parameters.

MATERIALS AND METHODS

Male and female CD-COBS rats (Charles River, Italy), 180–200 g were housed at constant temperature (23 °C) and humidity (60%) under fixed 12 h light and dark cycles for at least four days before the experiment. All the experiments were performed at the same time of day to avoid circadian variations in levels. The rats were killed by focussed microwave irradiation to the head (1·3 kW, 2·45 GHz for 4 s). The brain was removed quickly and dissected into different areas, i.e., striatum, hippocampus, hemispheric rest and midbrain-hindbrain as described by Valzelli & Garattini (1968) and the region containing the n. accumbens plus olfactory tubercle (NA-OT)

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as described by Koob et al (1975). The tissues were homogenized in a solution of 15% 1 M formic acid-85% acetone before proceeding to the measurement of acetylcholine (ACh) and choline by the radiochemical method of Saelens et al (1970) with modifications (Ladinsky et al 1976). Choline *O*-acetyltransferase activity was measured by a modified radiochemical method of McCaman & Hunt (1965). Acetylcholinesterase was determined by the method of McCaman et al (1968). Specific muscarinic receptor binding was measured by the method of Laduron et al (1979) using [^{3}H]dexetimide as the radioligand.

The rotarod test was used for measuring the muscle relaxant effect of the drug. The apparatus consists of a horizontal plastic rod, 12·8 cm in diameter rotating at 10 rev min^{-1}. Groups of 7–10 naive rats were first trained to keep their balance on it for more than two min. After different times of drug administration, the percentage of the rats that fell from the rod within 2 min was determined.

Buspirone HCl and apomorphine HCl were dissolved in water. Haloperidol was dissolved in 5 mM HCl and clozapine in 90 mM HCl. All the drugs were administered by the routes and at the doses and times shown in the Tables. The doses refer to buspirone and apomorphine as their salts and to haloperidol and clozapine as their free bases.

RESULTS

The administration of buspirone at 20 mg kg^{-1} orally induced a decrease in striatal ACh of about 25% in female rats. The effect was somewhat less marked in the n.accumbens-olfactory tubercle (NA-OT) (−10%) while it could not be observed in other discrete brain regions such as the hippocampus, hemispheric rest and the brainstem (Table 1). No changes in choline levels were detectable in the brain areas examined. The choline levels in control striatum, hippocampus, NA-OT, hemispheric rest and brainstem were, respectively, 18·5 ± 0·7. 15·8 ± 2·0, 31·3 ± 1·1, 15·5 ± 1·0 and 20·2 ± 1·0 nmol g^{-1} (n = 8).

Table 1. Effect of buspirone on acetylcholine content in female rat brain areas. Buspirone was administered orally at the dose of 20 mg kg^{-1} and the rats were killed after 60 min.

	Acetylcholine (nmol g^{-1})	
Brain area	Controls	Treated
Striatum	64·7 ± 2·3 (8)	49·3 ± 1·1 (8)[a]
Hippocampus	25·6 ± 0·5 (13)	25·4 ± 0·7 (13)
NA-OT	63·9 ± 1·1 (8)	57·3 ± 2·8 (8)[b]
Hemispheric rest	16·0 ± 0·5 (15)	15·1 ± 0·5 (15)
Brainstem	29·1 ± 1·5 (8)	26·3 ± 0·5 (8)

[a] $P < 0·01$. [b] $P < 0·05$ vs respective controls. Student's *t*-test was used for statistical analysis.

Table 2. Time-course effect of buspirone on acetylcholine levels in female and male rat striatum. Buspirone was administered orally at the dose of 20 mg kg^{-1}. Data are the means ± s.e.m. (n).

Time after buspirone (min)	Striatal acetylcholine (nmol g^{-1})	
	Females	Males
0	67·0 ± 1·7 (8)	66·8 ± 1·5 (14)
15	44·3 ± 1·6 (8)[a]	47·8 ± 2·6 (8)[a]
30	49·6 ± 3·0 (8)[a]	54·4 ± 2·0 (16)[a]
60	47·5 ± 4·0 (8)[a]	61·5 ± 1·2 (15)[b]
120	57·9 ± 0·9 (8)[a]	62·3 ± 1·0 (8)[a]
240	65·5 ± 1·6 (8)[a]	—

[a] $P < 0·01$. [b] $P < 0·05$ vs controls. Dunnett's test was used for statistical analysis.

The lowering effect of buspirone on ACh levels was more marked and longer lasting in the striatum of female than in male rats (Table 2). Also, the myorelaxant effect (rotarod test) of the drug was much more marked in duration and in intensity in female than in male rats. In females, buspirone was active for longer than 120 min at a dose of 20 mg kg^{-1} and for more than 48 h at a dose of 40 mg kg^{-1}; in contrast, in male rats, only the highest dose (40 mg kg^{-1}) was effective in producing muscle relaxation and this effect lasted less than 12 h (Table 3).

Table 4 shows that the decrease in striatal ACh induced by buspirone was dose-dependent, the effect

Table 3. Time course and dose-dependence of the effect of buspirone on rotarod performance in female and male rats. The animals were trained twice daily for three days before the experiment. Each group consisted of 7–10 animals.

	Rotarod failures (%)																	
Dose mg kg^{-1} (oral)	Females (time min or h)									Males (time min or h)								
	15	30	60	120	240	360	12 h	24 h	48 h	15	30	60	120	240	360	12 h	24 h	
5	50	12	0	—	—	—	—	—	—	0	—	0	0	—	—	—	—	
10	85	29	14	14	14	29	29	29	14	29	0	0	14	0	—	—	—	
20	100	66	88	100	22	22	22	22	—	0	28	28	14	0	—	—	—	
40	100	88	75	88	100	75	75	62	50	100	100	100	100	33	66	33	0	

Table 4. Dose-effect of buspirone on acetylcholine levels in female rat striatum 1 h after administration.

Buspirone mg kg^{-1}, oral	Striatal acetylcholine (nmol g^{-1})
0	66·8 ± 1·5 (14)
2·5	59·9 ± 2·0 (7)[b]
5·0	59·1 ± 1·0 (7)[a]
10·0	57·0 ± 1·4 (14)[a]
20·0	47·5 ± 1·3 (14)[a]
40·0	45·4 ± 1·4 (10)[a]

[a] $P < 0·01$. [b] $P < 0·05$ vs controls. Statistical analysis was by Dunnett's test.
Data are the means ± s.e.m. (n).

being evident at 2·5 mg kg^{-1}, orally. The maximum decrease of about 30% was produced at 20–40 mg kg^{-1}.

Other experiments indicated that buspirone had no direct effect in vitro on striatal choline acetyltransferase and acetylcholinesterase activites up to the concentration of 10^{-5} M. The enzyme activities of the 500 *g* supernatant fraction were 113 ± 6 μmol ACh syn. h^{-1} g^{-1} protein and 18·1 ± 0·9 mmol ACh hyd. h^{-1} g^{-1} protein, respectively.

The drug showed weak ability to displace [^{3}H]dexetimide from its specific muscarinic cholinergic receptor binding sites in striatum, its IC50 being 6×10^{-5} M compared with the respective IC50's of atropine and clozapine of $6·8 \times 10^{-9}$ M and $2·5 \times 10^{-8}$ M.

Repeated treatment with buspirone for seven days resulted in a loss (homologous tolerance) of the lowering effect on striatal ACh by an acute challenge with buspirone (Table 5) but did not affect its IC50 in displacing [^{3}H]dexetimide binding. Homologous tolerance was also produced to haloperidol although statistical significance was reached only after 28 days (Table 5). In contrast, no homologous tolerance to clozapine, an atypical non-cataleptogenic neuroleptic, was induced even after 28 days (Table 5). The lack of development of this phenomenon may be attributable to the anticholinergic property of clozapine which in some way may counteract the effects of dopamine receptor blockade.

Table 6. The cross tolerance response to buspirone and clozapine by rats treated long term with haloperidol or buspirone.

Treatment		Striatal acetylcholine
Chronic	Acute	(nmol g^{-1})
Vehicle	Vehicle	65·8 ± 2·2
Vehicle	Buspirone	49·4 ± 1·8[a]
Vehicle	Clozapine	44·5 ± 1·9[a]
Haloperidol	Vehicle	74·6 ± 3·0
Haloperidol	Buspirone	70·3 ± 1·8
Haloperidol	Clozapine	51·6 ± 1·2[a,b]
Buspirone	Clozapine	45·4 ± 2·3[a,b]

Statistical analysis was by ANOVA (2 × 2) factorial analysis, Tukey's test and Tukey's test for unconfounded means.
Long-term treatments: haloperidol 1 mg kg^{-1} i.p. twice daily for 28 days, 48 h withdrawal; buspirone, 20 mg kg^{-1} oral, twice daily for 10 days, 24 h withdrawal. Acute doses: buspirone 20 mg kg^{-1} oral, 60 min; clozapine 20 mg kg^{-1} i.p., 60 min.
[a] = $P < 0·01$ vs vehicle + vehicle.
[b] = $P < 0·01$ vs haloperidol + vehicle.
Data are means ± s.e.m. (8).

In cross tolerance experiments, rats chronically treated with haloperidol did not respond to buspirone while they were still sensitive to the lowering effect of clozapine on striatal ACh (Table 6). Similarly, animals treated chronically with buspirone responded equally well to the lowering dose of clozapine.

To find whether the effect of buspirone on striatal ACh was related to the dopaminergic system, its inhibitory effect on apomorphine-induced increase in striatal ACh was tested. As indicated in Table 7, only a high dose of the drug (80 mg kg^{-1}, orally), was capable of completely inhibiting the effect of apomorphine.

DISCUSSION

Buspirone selectively decreased the level of ACh in the striatum and this pattern of activity is reminiscent

Table 5. Effect of long term treatments with buspirone, haloperidol and clozapine on striatal acetylcholine content in female rats.

Drug in columns B, C and D	Treatment days	Striatal acetylcholine A: Vehicle + Vehicle (chronic / acute)	B: Vehicle + Drug (chronic / acute)	C: Drug + Vehicle (chronic / acute)	D: Drug + Drug (chronic / acute)	Interaction[b]
Buspirone	7	64·5 ± 2·7 (8)	46·4 ± 1·6 (8)[a]	71·0 ± 2·2 (8)	67·7 ± 1·7 (8)	F 1,28 = 12·0 $P < 0·01$
Haloperidol	11	68·2 ± 2·5 (8)	42·8 ± 3·0 (8)[a]	73·0 ± 3·0 (8)	55·6 ± 1·4 (8)	NS
Haloperidol	28	62·8 ± 2·0 (9)	40·6 ± 2·4 (9)[a]	68·6 ± 2·2 (9)	59·2 ± 2·9 (9)	F 1,32 = 6·8 $P < 0·05$
Clozapine	28	63·1 ± 2·0 (10)	40·4 ± 0·9 (10)[a]	73·0 ± 2·0 (10)[a]	42·3 ± 2·3 (10)[a]	NS

Long term double treatments: buspirone 20 mg kg^{-1}, oral, 24 h withdrawal; haloperidol, 1 mg kg^{-1}; i.p. 48 h withdrawal; clozapine, 20 mg kg^{-1} i.p. 48 h withdrawal. Acute challenge: buspirone, 20 mg kg^{-1} oral, 60 min; haloperidol, 0·5 mg kg^{-1} i.p.; clozapine, 20 mg kg^{-1} i.p., 60 min.
a = $P < 0·01$ vs vehicle + vehicle group.
b = ANOVA (2 × 2) factorial analysis and Tukey's test for unconfounded means. Data are means ± s.e.m (n).

Table 7. Effect of buspirone on the apormorphine (1·5 mg kg^{-1} i.p.) induced increase in the striatal acetylcholine levels of female rats.

Drug (mg kg^{-1} oral)	Striatal acetylcholine (nmol g^{-1}) Saline	Apomorphine	Interaction
A			
Saline	68·9 ± 0·9	78·2 ± 2·5[b]	NS
Buspirone, 20	48·5 ± 2·5[a]	54·0 ± 1·7[a]	
B			
Saline	68·2 ± 1·6	83·0 ± 3·1[a]	F 1,28 = 9·24
Buspirone, 80	44·5 ± 1·2[a]	46·4 ± 2·2[a]	$P < 0·01$

Statistical analysis was performed by ANOVA (2 × 2) factorial analysis, Tukey's test and Tukey's test for unconfounded means.
[a] $P < 0·01$, [b] 0·05 vs respective saline controls.
The animals were killed 60 min after buspirone and 30 min after apomorphine.
The data are means ± s.e.m. (8).

of the action of dopaminergic drugs (Consolo et al 1975). Both the biochemical and pharmacological effects of buspirone were longer-lasting in female than in male rats, and this discrepancy may be due to a difference in the metabolism of buspirone by the two sexes.

The mechanism involved in the lowering effect of the drug is not fully elucidated although some mechanisms may be excluded. For example, the action of buspirone does not seem to be related to a direct effect on the enzymes involved in the synthesis and hydrolysis of ACh. In addition, the lowering of striatal ACh is unlikely to be due to a direct reaction with muscarinic receptors since displacement of [^{3}H]dexetimide occurred only at a concentration about 10 000 times higher than that of an antimuscarinic agent such as atropine. The data also show that the effect of buspirone is only feebly related to an inhibition of the dopaminergic system as evidenced by its low potency as a blocker of apomorphine's increase in striatal ACh. In this regard, buspirone behaved more like the atypical neuroleptic, clozapine (Consolo et al 1975), and greatly differed from haloperidol or pimozide, both potent dopamine receptor blockers which at low doses are known to inhibit apomorphine's cholinergic effect (Consolo et al 1975). These neurochemical findings are consistent with pharmacological data suggesting that buspirone is not a typical neuroleptic agent. In addition, in vitro binding experiments in the presence and in the absence of guanosine triphosphate indicate that buspirone inhibits [^{3}H]*N*-propylnorapomorphine (NPA) binding in a manner similar to dopamine agonists such as apomorphine, NPA and piribedil (Stanton et al 1981). These authors suggest that buspirone may act as an agonist at certain presynaptic dopaminergic receptors. Such a mechanism could conceivably provoke a decrease in striatal ACh content, as the inhibition of dopamine release caused by stimulation of presynaptic dopamine receptors may lead to a disinhibition of the postsynaptic cholinergic interneurons and to a release of ACh.

The biochemical mechanism of the antianxiety action of buspirone is unclear. Laboratory studies in vivo and in vitro do make it clear, however, that buspirone does not act through mechanisms common to benzodiazepines or GABA (Stanton et al 1981). Also the action of buspirone on brain cholinergic parameters differs completely from that of the benzodiazepines (Ladinsky et al 1981).

The data reported thus support the notion that buspirone is a novel psychotropic drug for which more extensive neurochemical studies may be desirable.

Acknowledgements

Buspirone HCl was kindly supplied by Bristol-Myers Co, New York.

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