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ACTION OF S 1432, A NEW PSYCHOTROPIC DRUG, ON THE CENTRAL CHOLINERGIC SYSTEM

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Abstract—S 1432 (hydroxyethyl-9', purinyl-6'-1-benzhydryl-4-piperazine dihydrochloride), an anticonvulsant, muscle relaxant and CNS depressant in experimental animals, increased the acetylcholine level in the striatum (50%) hippocampus (25%) and mesencephalon (15%) but not in the diencephalon or hemispheric residuum of the rat at doses of 20 and 40 mg/kg, i.p. The effect lasted for about 120 min after a single dose of 40 mg/kg. The drug markedly decreased the choline level in the striatum but not in the other brain areas observed. The action of S 1432 on striatal acetylcholine was not blocked by pimozide indicating that the increase was not modulated through dopaminergic neurons. Furthermore, the drug did not alter whole brain or brain area levels or the turnover of noradrenaline, serotonin or dopamine although there was a tendency towards a decrease in the latter. Pretreatment with S 1432 prevented pentylenetetrazol convulsions in 11/12 rats. Under these conditions, pentylenetetrazol completely antagonized the effect of S 1432 on striatal but not hippocampal acetylcholine. Thus, similarly to diazepam (14), a biochemical interaction of S 1432 with pentylenetetrazol is demonstrated.

S 1432 [Hydroxyethyl-9', purinyl-6'-1-benzhydryl-4-piperazine dihydrochloride] (Fig. 1) is a new drug provided with the properties of an anticonvulsant, a muscle relaxant and CNS depressant in experimental animals (unpublished data from the Servier Laboratories, Paris). Furthermore, the compound does not affect either the cardiovascular or the autonomic nervous system and is devoid of hypnotic and neuroleptic activity.

The pharmacological spectrum in general indicates that S 1432 may be useful clinically as an antianxiety drug. It was thus of interest to characterize this new agent at the neurochemical level and to compare it with the benzodiazepines, a class of minor tranquilizers with which it shares many pharmacological properties.

MATERIALS AND METHODS

Female CD rats (Charles River) weighing 215–225 g were housed at 22° with standardized light cycles (12 hr of light and 12 hr of darkness) for at least 4 days prior to experimentation. For the estimation of brain area acetylcholine the animals were killed by decapitation and the head was immediately immersed in liquid nitrogen for 6–7 sec. The brain was removed from the cranium as rapidly as possible and the left corpus striatum and other brain areas (mesencephalon, diencephalon, cerebral hemispheres, cerebellum and hippocampus) were isolated under *n*-pentane at −5° following the technique of Campbell and Jenden [1]. The brain parts were frozen in liquid nitrogen, weighed in the frozen state and pulverized [2]. The radiochemical method of Saelens, Allen and Simke [3] was then used for the measurement of acetylcholine and choline with a modification described earlier [4]. The results are expressed as nmoles free base of acetylcholine and choline per g tissue wet weight.

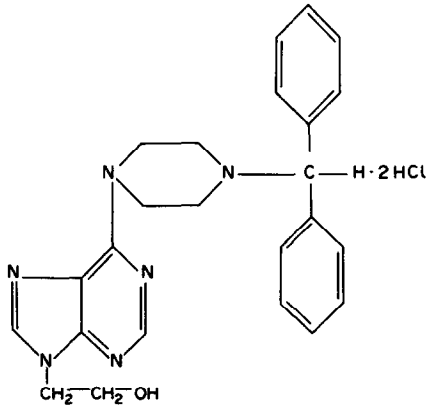

Fig. 1. Chemical structure of S 1432.

Choline *o*-acetyltransferase activity was determined by the radiochemical method of McCaman and Hunt [5] with some modifications [6] and cholinesterase activity was determined by the radiochemical method of McCaman, Tomey and McCaman [7].

3-Methoxy-4-hydroxy-phenylethyleneglycol sulfate (MOPEG-SO_4) was measured by the method of Meek and Neff [8] in whole brain. Indoles were estimated according to the method of Giacalone and Valzelli [9].

Dopamine, noradrenaline and homovanillic acid were determined in the striatum by the fluorimetric methods of Laverty and Taylor [10] and Korf *et al.* [11, 12]. The kinetic constants were calculated according to the method of Brodie *et al.* [13].

S 1432 was kindly supplied by the Servier Laboratories, Paris and dissolved in distilled water.

RESULTS

Table 1 shows the effect of S 1432, 40 mg/kg, on the acetylcholine and choline level in the cerebellum,

Table 1. Effect of S 1432 on the acetylcholine and choline level in rat brain areas

Brain area	Acetylcholine (nmoles/g wet wt ± S.E.)		Choline (nmoles/g wet wt ± S.E.)	
	Controls	Treated	Controls	Treated
Hemispheric rest‡	9.7 ± 1.0 (4)	10.6 ± 0.6 (4)	49.7 ± 1.8 (4)	47.3 ± 2.8 (4)
Hippocampus	18.0 ± 1.0 (6)	22.8 ± 1.0 (6)†	77.4 ± 3.6 (4)	71.1 ± 4.2 (4)
Striatum	28.5 ± 2.7 (4)	42.0 ± 1.9 (4)†	104.9 ± 4.3 (4)	64.4 ± 1.7 (4)†
Mesencephalon	17.1 ± 0.8 (4)	19.4 ± 0.5 (5)*	55.8 ± 3.6 (7)	59.9 ± 2.5 (4)
Diencephalon	19.8 ± 0.8 (9)	21.6 ± 1.0 (7)	45.6 ± 0.8 (4)	45.8 ± 1.8 (5)

* $P < 0.05$ Student's t-test.
† $P < 0.01$ Student's t-test.
‡ Cerebral hemispheres less striatum and hippocampus.
The rats were sacrificed 30 min after the administration of S 1432, 40 mg/kg, i.p. The figures in brackets represent the number of animals used.

Table 2. Effect of S 1432 at 20 and 40 mg/kg on the acetylcholine level in rat brain areas

Treatment	Dose mg/kg i.p.	Acetylcholine (nmoles/g wet wt ± S.E.)		
		Hippocampus	Striatum	Mesencephalon
Controls	—	18.0 ± 1.0 (6)	28.9 ± 1.3 (12)	17.1 ± 0.5 (10)
S 1432	20	20.2 ± 0.5 (6)	35.9 ± 1.7 (7)*	19.3 ± 0.6 (7)*
S 1432	40	22.8 ± 1.0 (6)*	44.7 ± 2.7 (12)*	19.5 ± 0.6 (7)*

* $P < 0.01$ Dunnett's test.
The rats were sacrificed after 30 min. The figures in brackets represent the number of animals used.

Table 3. Time-course effect of S 1432 on the acetylcholine level in rat hippocampus and striatum

Treatment	Time (min)	Acetylcholine (nmole/g wet wt ± S.E.)	
		Hippocampus	Striatum
Controls	—	18.1 ± 0.5 (12)	29.8 ± 1.4 (10)
S 1432	30	22.8 ± 1.0 (6)*	45.1 ± 2.4 (11)*
S 1432	60	22.5 ± 0.6 (6)*	49.1 ± 3.5 (6)*
S 1432	120	22.3 ± 1.0 (7)*	44.4 ± 4.5 (6)*
S 1432	240	20.4 ± 0.9 (7)	30.5 ± 0.5 (4)

* $P < 0.01$ Dunnett's test.
S 1432 was administered at a dose of 40 mg/kg. The figures in brackets represent the number of animals used.

Table 4. Effect of pimozide or pentylenetetrazol on the S 1432- induced increase in the acetylcholine level in the rat striatum and hippocampus

Drug in C and D	A Controls	B S-1432	C Drug	D drug + S 1432	Brain area
Pimozide†	30.6 ± 0.9 (6)	42.1 ± 2.3 (6)*	11.1 ± 0.5 (6)*	26.4 ± 1.2 (6)	Striatum
Pentylenetetrazol‡	29.5 ± 0.8 (5)	50.1 ± 4.0 (5)*	27.9 ± 0.8 (5)	31.7 ± 1.6 (5)	Striatum
Pentylenetetrazol‡	18.5 ± 0.8 (6)	23.7 ± 1.1 (6)*	12.5 ± 1.5 (6)*	15.8 ± 0.9 (6)	Hippocampus

Statistics: Anova (2 × 2) factorial analysis and Tukey's test for unconfounded means. No interaction between pimozide and S 1432. Interaction between pentylenetetrazol and S 1432 in the striatum.
* $P < 0.01$ vs. respective controls.
† Pimozide was administered i.p. at a dose of 1 mg/kg and S 1432 at a dose of 40 mg/kg, i.p. The animals were killed 240 min after pimozide and 30 min after S 1432.
‡ Pentylenetetrazol was administered i.p. at a dose of 80 mg/kg and S 1432, 40 mg/kg, i.p. The animals were killed 60 min after S 1432 and 12 min after pentylenetetrazol.

Table 5. Effect of various doses of S 1432 on the brain level of 5-HT, 5-HIAA, MOPEG-SO_4 and HVA

Treatment	Dose (mg/kg i.p. 60 min)	5-HT (μg/g)	5-HIAA (μg/g)	MOPEG-SO_4 (μg/g)	HVA (μg/g)
Controls	—	0.26 ± 0.02	0.36 ± 0.03	154.0 ± 5.46	0.42 ± 0.09
S 1432	10	0.30 ± 0.02	0.34 ± 0.01	148.8 ± 5.44	—
S 1432	20	0.27 ± 0.01	0.36 ± 0.01	163.3 ± 4.16	0.41 ± 0.03
S 1432	30	0.30 ± 0.01	0.34 ± 0.01	—	—
S 1432	40	0.31 ± 0.01	0.37 ± 0.02	169.8 ± 4.88	0.24 ± 0.09*

Each figure represents the mean of 6 animals.
* $P < 0.01$ Dunnett's Test was used for statistical analysis.

Table 6. Effect of S 1432 on the turnovers of dopamine in the striatum and noradrenaline in the brainstem

Treatment	Steady state level (μg/g ± S.E.)	Rate of disappearance $K(hr^{-1})$ ± S.E.	Turnover rate (μg/g/hr)	Turnover time (hr)
Dopamine in striatum				
Controls	5.51 ± 0.6 (4)	0.21 ± 0.02	1.16	4.76
S 1432	4.54 ± 1.2 (4)	0.16 ± 0.07	0.73	6.25
Noradrenaline in brainstem				
Controls	0.38 ± 0.02	0.23 ± 0.04	0.087	4.35
S 1432	0.37 ± 0.02	0.29 ± 0.04	0.109	3.45

S 1432 was administered at a dose of 20 mg/kg, i.p. The rats were killed 60 min later.

diencephalon, mesencephalon, hippocampus and hemispheric residuum (less striatum and hippocampus) at 30 min after intraperitoneal administration. Among the hemispheric structures, the acetylcholine level was increased only in the striatum (47%) and hippocampus (27%) and not in the hemispheric residuum. The level was also slightly but significantly increased in the mesencephalon (13%).

The choline level was significantly decreased in the striatum by 39% and remained unaltered in the other areas considered at this dose and time after the administration of S 1432.

The effect of S 1432 at 20 and 40 mg/kg on the acetylcholine level in the striatum, hippocampus and mesencephalon at 30 min after administration is shown in Table 2. The acetylcholine level in the hippocampus was not significantly changed at the dose of 20 mg/kg but was increased by 27 per cent at 40 mg/kg. The striatal acetylcholine level was increased by 24% at 20 mg/kg and by 55% at 40 mg/kg. In the mesencephalon, the increase of the quaternary amine was of about the same order (13–14%) at both doses used.

It thus appears that the striatum is the area most sensitive to the action of S 1432 on acetylcholine.

A time-course of the effect of S 1432, 40 mg/kg (Table 3), indicates that the maximal increase in both striatal and hippocampal acetylcholine was obtained between 30 and 120 min and the level returned to normal by 240 min after administration.

The prior administration of pimozide, a powerful and selective central dopamine receptor blocker, did not antagonize the action of S 1432 on striatal acetylcholine (Table 4). In fact, in the pimozide pretreated animals (1 mg/kg, i.p., 240 min), in which the striatal acetylcholine level was markedly lowered, S 1432 produced approximately the same net increase in acetylcholine (from 30.6 ± 0.9 to 42.1 ± 2.3 nmoles/g in the group with S 1432 alone, and from 11.1 ± 0.5 to 26.4 ± 1.2 nmoles/g in the group with pimozide plus S 1432). As shown by statistical analysis, these data indicate that the action of S 1432 is not mediated through the dopaminergic system.

On the other hand, the increase in striatal acetylcholine produced by S 1432 was completely antagonized by the post-administration of pentylenetetrazol (80 mg/kg, i.p., 12 min) whereas there was no interaction between these drugs in the hippocampus. By itself, this convulsant did not affect the acetylcholine level in the striatum but markedly decreased the level in the hippocampus (Table 4). S 1432 pretreatment prevented pentylenetrazol convulsions in 11/12 rats under the conditions described in Table 4.

Striatal choline *o*-acetyltransferase and cholinesterase activities were not affected by the *in vivo* incubation of the 500 g supernatant fraction with S 1432 at concentrations of 5 μg/2 mg tissue/ml and 2 μg/0.3 mg tissue/ml, respectively. The enzymic activities were 20.1 ± 0.28 μmoles acetylcholine formed/hr/g wet wt and 3268 ± 131 μmoles acetylcholine hydrolyzed/hr/g wet wt, respectively.

Whole brain levels of 5-HT, 5-HIAA and MOPEG-SO_4 were not altered by treatment with S 1432, 10–40 mg/kg, 60 min (Table 5). Furthermore, at a dose of 20 mg/kg, the drug did not affect the level of striatal HVA but at 40 mg/kg, it produced a significant decrease in the level of this dopamine metabolite of about 40% (Table 5).

Neither the turnover of NE and its steady state level in the brainstem, nor the turnover of DA and its state level in the striatum were altered by S 1432, 20 mg/kg (Table 6). There was, however, a tendency towards a decrease in the turnover of DA.

DISCUSSION

We have shown that S 1432 exerts its action of increasing acetylcholine mainly in subcortical structures such as the hippocampus and the striatum. Furthermore, this drug does not induce its effect on acetylcholine via a direct action on the metabolism of this quaternary amine since the enzymes involved in the synthesis and hydrolysis of acetylcholine were apparently not altered.

Since only few biochemical data are available on the action of S 1432 on other neuronal systems, it cannot yet be established whether the mechanism by which this drug affects cholinergic neurons is direct or indirect through other putative neurotransmitters. However, it may be inferred from the overall negative biochemical effect of S 1432 (at 20 mg/kg) on the dopaminergic system and the ineffectiveness of dopamine receptor blockade to alter the action of S 1432 on striatal acetylcholine, that the action of this new tranquilizer is not mediated through the dopaminergic system. Furthermore, the lack of effect of S 1432 on the level of serotonin and 5-HIAA and MOPEG-SO_4 tends to suggest that also the serotoninergic and catecholaminergic systems are not involved in this biochemical action of S 1432.

The compound S 1432 is about 5 times less potent than diazepam [14] in increasing the acetylcholine level in the striatum and it could be qualitatively distinguished from the benzodiazepines in a few aspects of its action. In contrast to diazepam, S 1432 did not affect the acetylcholine level in the hemispheric residuum [14] but on the other hand had a profound action on the choline level in the striatum. This decrease in striatal choline is reminiscent of the action of the anticonvulsant drug carbamazepine [15].

Finally, whereas pentylenetetrazol gave an unusual interaction with diazepam, producing a decrease in the striatal acetylcholine level below that of the control [14], S 1432 was completely blocked by pentylenetetrazol.

In general, the pharmacological similarities of S 1432 and diazepam are reflected also in their sharing several, but not all, biochemical properties on the central cholinergic system.

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