ANALYSIS OF THE HYPOTHERMIC ACTION OF 8-HYDROXY-2-(DI-N-PROPYLAMINO)-TETRALIN, AND OF 1-(2-PYRIMIDINYL)-PIPERAZINE AND ITS DERIVATIVES

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8-Hydroxy-2-(di-propylamino)-tetralin (8-OH-DRAT) is a serotonin agonist. It binds specifically, with high affinity (K_d = 4 nM), with brain membrane fragments [5] and counteracts binding of ^{3}H-serotonin competitively (K_i = 8.67 ± 0.03) [7]. Its most important effects — anxiolytic, ability to increase locomotion and to induce a "serotonin syndrome," hypothermia, and hyperphagia — are linked with activation of 1A-serotonin receptors [2, 4, 9]. Although buspirone, ipsapirone, gepirone, and other derivatives of 1-(2-pyrimidinyl)-piperazine (1-PP), structurally different from 8-OH-DRAT, in radioligand investigations exhibit analogous properties [6, 7], they do not necessarily reproduce all the effects of 8-OH-DRAT [3, 10]. The reasons for these differences are not clear.

The paper describes an analysis of the hypothermic effect of 1-PP and its derivatives, by comparison with the analogous effect of 8-OH-DRAT.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 160-200 g and mice weighing 18-22 g. The test substances were injected subcutaneously in increasing doses: 0.1 and 0.3, 1.3 and 10 mg/kg. The minimal effective dose (MED), on injection of which the body temperature fell significantly ($p < 0.05$) after 30 min compared with the control, was determined. Body temperature was measured in the rectum 30, 60, and 90 min after injection of the substances, in an ambient temperature of 19-22°C. Buspirone, ipsapirone, and 8-OH-DRAT were generously provided by the firm "Troponwerke" (West Germany); 1-PP, campirone, caplapirone, and levopirone were synthesized in the Institute of Physico-Organic Chemistry and Carbon Chemistry, Academy of Sciences of the Ukrainian SSR.

The hypothermic effects of these substances were analyzed by preliminary injection of *p*-chlorophenylalanine (PCPA) into mice in a dose of 300 mg/kg, intraperitoneally, on two consecutive days in order to exhaust the reserves of the mediator in the serotoninergic brain neurons [8]. The hypothermic activity of the substances in rats was compared with previous data [1] on their effect (in a concentration of 10^{-5} mole/liter) on ^{3}H-serotonin (^{3}H-HT) from electrically stimulated slices of the midbrain nuclei raphe and slices of cerebral cortex (of rats), preincubated with the labeled amine. The effect of (±)-alprenolol and (±)-propranolol (ICI, Great Britain) on drug-induced hypothermia also was studied in experiments on rats, bearing in mind that both substances had high affinity (K_{iO} = 6.5-7.3) for 1A and 1B serotonin receptors [6]. The latter drugs were injected intraperitoneally in doses of 10 and 30 mg/kg immediately after, and in a dose of 15 mg/kg 30 min after subcutaneous injection of the test substances. Each series of experiments was performed on 8-16 animals. Confidence intervals of the means were determined at the p = 0.05 level. The rank correlation coefficient was calculated by Spearman's method.

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TABLE 1. Hypothermic Effect Induced by 8-OH-DRAT, 1-PP, and Its Derivatives in Rats and Mice

Substance	Dose, mg/kg	Changes in body tempeature (°C ± $S_{\bar{x}} \cdot t$)			
		30 min	60 min	30 min	60 min
		rats		mice	
Control	0	—0,5±0,42	—0,3±0,44	—0,4±0,15	—0,8±0,22
8-OH-DRAT	0,1	—1,2±0,81	—1,1±0,56*	—0,7±0,35	+0,1±0,44
	0,3	—1,4±0,61*	—1,4±0,83*	—1,3±0,61*	—1,2±0,80
	1,0	—3,4±0,64*	—3,3±0,51*	—2,5±0,62*	—2,5±0,59*
1-PP	0,3	+0,1±0,15	—0,2±0,32	n.t	
	1,0	—1,0±0,64	—0,6±0,49	—0,4±0,33	—0,8±0,49
Buspirone	3,0	—1,6±0,91*	—1,6±0,78*	—1,2±0,57*	—1,2±0,92
	10,0	—2,1±0,43*	—1,9±0,88*	—1,1±0,63*	—0,9±0,81
	0,3	n.t.		—0,9±0,65	—1,1±0,67
Ipsapirone	1,0	—0,7±0,37	—0,7±0,40	—1,2±0,44*	—1,3±0,42
	3,0	—1,2±0,42*	—1,0±0,54*	—1,5±0,55*	—1,4±0,45*
	1,0	+0,2±0,30*	+0,2±0,15	—0,3±0,60	—0,5±0,72
	3,0	—0,5±0,47	—0,4±0,40	—1,4±0,74*	—0,6±0,27
	10,0	—1,3±0,32*	—1,8±0,61*	—1,7±1,06*	—2,2±1,02*
Campirone	0,3	—0,4±0,30	+0,1±0,51	—0,6±0,56	—0,4±0,65
	1,0	—1,8±0,51*	—1,5±0,56*	—1,0±0,49*	—1,5±0,79
	3,0	—2,7±0,66*	—3,0±0,71*	—2,3±1,03*	—2,3±0,76*
	10,0	—3,0±0,76*	—3,9±1,21*	n.t.	
Caplapirone	0,3			—0,3±0,69	—0,4±0,93
	1,0	+0,3±0,33*	+0,4±0,40	—0,7±0,52	—0,6±0,49
	3,0	+0,5±0,41*	+0,7±0,40*	—0,8±0,81	—1,0±0,65
	10,0	+0,1±0,40	—0,1±0,31	—1,5±0,52*	—1,2±0,83
Levopirone	0,3	+0,3±0,28*	+0,4±0,28*	—0,4±0,94	—0,6±0,99
	1,0	—1,3±0,33*	—0,7±0,33	—1,7±0,85*	—1,8±1,08*
	3,0	—2,0±0,60*	—1,5±0,71*	—2,2±0,77*	—1,9±0,75*
	10,0	—2,9±0,46*	—2,9±0,80*	n.t.	

Legend. *) differences from control statistically significant ($p < 0.05$). n.t.) effect of these doses not tested.

EXPERIMENTAL RESULTS

The test compounds led to dose-dependent lowering of the body temperature of rats and mice. Caplapirone, which lowered the body temperature in mice but not in rats, only in a dose of 10 mg/kg, and ipsapirone, which was effective in rats also in this dose only, were exceptions. The strongest hypothermic action was exhibited by 8-OH-DRAT, which was active in doses of 0.1-0.3 mg/kg (Table 1). The order in which the substances are arranged in order of diminishing hypothermic activity in rats and mice shows high correlation: $r = 0.95$. This suggests that the cellular and neurochemical mechanisms of the hypothermic effect of the test substances are identical in the two species of rodents.

Preliminary injection of PCPA reduced the hypothetic effect of 8-OH-DRAT, buspirone, and campirone but did not change hypothermia induced by 1-PP (Fig. 1). Normal functioning of serotoninergic brain neurons is evidently essential for manifestation of the hypothermic effect not only of 8-OH-DRAT [4], but also of buspirone-like drugs.

The hypothermic activity of the test substances correlated satisfactorily with (Table 2) the degree of their inhibitory influence on ^{3}H-serotonin release by electrically stimulated slices of nuclei raphe ($r = 0.84$), but not by slices of cerebral cortex ($r = 0.66$), although reduction of functional activity of serotoninergic neurons occurred in both cases: in the first, through activation of somatodendritic 1A-autoreceptors and inhibition of spike activity of these neurons by the substances [1, 11], second, through activation of 1B-autoreceptors of axon terminals and inhibition of pulsed release of the mediator. This raises doubts about the correlation between the hypothermic effect of 8-OH-DRAT, buspirone, and similar substances and depression of function of HT-ergic neurons, more especially because PCPA, which also depresses their functional activity by exhausting reserves of the mediator, does not cause hypothermia: the body temperature of the mice before and after injection of PCPA in a total dose of 600 mg/kg was 36.7 ± 0.24 ($n = 323$) and 36.9 ± 0.23°C, respectively ($n = 32$). PCPA also counteracts hypothermia induced by 8-OH-DRAT and by buspirone temporarily — only for the first 30 min (Fig. 1).

All these observations are more in harmony with the view that the hypothermic effect of 8-OH-DRAT, buspirone, and its structural analogs is associated predominantly with activation of postsynaptic 1A-receptors.

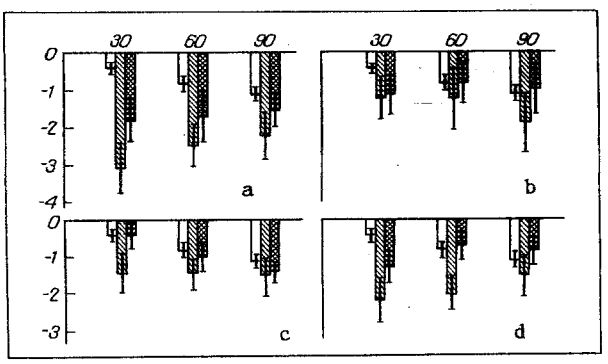

Fig. 1. Effect of preliminary loading of mice with *p*-chlorophenylalanine on hypothermic effect of 8-OH-DRAT (a), 1-PP (b), buspirone (c), and campirone (d). Abscissa, time after injection of substance (30, 60, and 90 min); ordinate, change in body temperature, °C. Unshaded columns — control (n = 32), shaded columns — effect of 8-OH-DRAT (1 mg/kg), 1-PP, buspirone, and campirone (3 mg/kg); cross-hatched columns indicate effect of drugs in the same doses after two days of injection of PCPA (n = 16). Confidence intervals at p = 0.05.

TABLE 2. Comparison of Hypothermic Activity of 8-OH-DRAT, 1-PP, and Its Derivatives with Their Ability to Inhibit Pulsed Release of ^{3}H-Serotonin by Rat Brain Slices

Substance	Hypothermic activity, MED, mg/kg		Reduction (per cent of control $\bar{x} \pm S_{\bar{x}} \cdot t$) of ^{3}H-serotonin release by slices of*	
	rats	mice	dorsal nuclei	cerebral cortex
8-OH-DRAT	0,3 (1)	0,3 (1)	54±3 (2)	24±5 (3)
Buspirone	1 (2)	3 (3)	65±4 (1)	48±3 (1)
Levopirone	1 (2)	1 (2)	64±3 (1)	51±6 (1)
Campirone	1 (2)	1 (2)	58±4 (2)	27±3 (3)
Ipsapirone	3 (3)	10 (4)	52±4 (3)	10±8 (4)
1-PP	3 (3)	3 (3)	43±4 (4)	29±4 (3)
Caplapirone	10 (4)	Not active	39±10 (4)	36±5 (2)

Order of activity of substances given in parentheses.

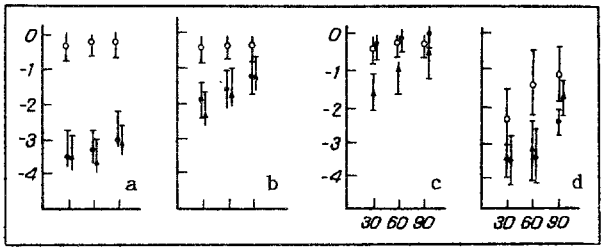

Fig. 2. Effect of (±)-propranolol on hypothermia induced in rats by 8-OH-DRAT (a), buspirone (b), ipsapirone (c), and campirone (d). Abscissa, time after injection (30, 60, and 90 min); ordinate, change in body temperature, °C. Propranolol in a dose of 10 mg/kg (a-c) or 30 mg/kg (d) was injected intraperitoneally immediately after subcutaneous injection of the substances in a dose of 1 mg/kg (a-c) or 3 mg/kg (d). Empty circles — water + propranolol, filled circles — substance + water, triangles — propranolol + substance. Confidence intervals at p = 0.05.

However, this view is contradicted by the fact that propranolol and alprenolol, which are regarded as blockers of 1A-serotonin receptors [4, 9], have little or no influence on the hypothermic effect of the serotonin agonists tested. If injected simultaneously with or 30 min after subcutaneous injection of 8-OH-DRAT, buspirone, campirone, and levopirone into rats, (±)-propranolol did not change their hypothermic effects, and they actually potentiated ipsapirone-induced hypothermia (Fig. 2). If (±)-alprenolol was injected 30 min after 8-OH-DRAT, buspirone, and levopirone, it reduced the effect of levopirone significantly, but only at the 90th minute. In a dose of 30 mg/kg, in which (±)-propranolol itself induces marked hypothermia, it weakened hypothermia due to levopirone and campirone (Fig. 2d) at the 90th minute and did not act addictively with time.

Thus hypothermia induced by 8-OH-DRAT, buspirone, and its structural analogs (but not 1-PP), in its initial period, is evidently due to activation of somatodendritic autoreceptors of serotoninergic neurons, but is to a greater degree dependent on their influence on postsynaptic 1A-serotonin receptors, on which these substances have a dual action as partial agonists. The properties of the partial antagonist are particularly marked in the case of ipsapirone and least of all with levopirone.

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