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Effect of Trazodone on Oxidative Metabolism of Rat Brain *in vitro*

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Oxygen uptake of rat brain homogenate was reduced by 1 mM trazodone, a new atypical antidepressant. Na,K-ATPase activity and the associated oxygen consumption of rat brain slices were also reduced. Oxygen consumption of rat brain slices was enhaced by dopamine and this effect was blocked by 0.0001 mM trazodone. This drug uncoupled oxidative phosphorylation.

Key words: ATP-ase activity, Oxygen uptake, P:O ratio, Trazodone.

Trazodone, 2-[3-[4-(chlorophenyl)-1-piperazinyl]propyl] 1,2,4,-triazolo [4,3-alpha] pyridin-3 (2H)-one, is a new antidepressant drug whose pharmacology has been studied by BRODGEN *et al.* (1) and SILVESTRINI *et al.* (14). The effects of trazodone on brain oxidative metabolism have not yet been investigated. In this paper the effects of trazodone on oxygen consumption (QO_2), glucose consumption and ATPase activity of rat brain *in vitro*, and the effect on dopamine-induced increase of respiration of rat brain slices are described.

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Materials and Methods

Brain slices and homogenates were prepared from male albino rats (150-200 g body wt) as previously described (16). The slices were incubated in a Krebs-Ringer phosphate medium (pH = 7.4) containing 10 mM glucose and either 5 mM or 105 mM potassium, or using the same medium with 5 mM K^+ but without calcium. Whole brain homogenates were prepared at 2-4°C in a Elvejhem-Potter, the incubation medium being (in M): 0.14 sucrose, 0.012 phosphate buffer, pH = 7.4, 0.002 sodium malate, 0.002 sodium pyruvate, 0.0048 magnesium chloride, 0.0012 sodium-ATP, 0.05 glucose and 0.05 mg hexo-

kinase. The mitochondria of rat brain were obtained by the CLARK and NIKLAS method (3), the incubation medium being (in M): 0.05 sucrose containing 0.0024 sodium ATP; 0.006 sodium malate; 0.006 sodium pyruvate; 0.012 tricine (N-tris- (hydroxymethyl) methylglycin) buffer, pH = 7.4; 0.016 phosphate buffer, pH = 7.4; 1.2×10^{-5} cytochrome C; 0.0004 magnesium sulphate; 0.012 sodium fluoride; 0.02 glucose; and 0.02 mg hexokinase.

The final volume of single flasks was 2.7 ml in the different experiments.

Oxygen uptake was determined by a direct manometric technique (15). Glucose uptake was estimated by measuring the glucose concentrations in the medium at the end of the incubation period, by the glucose-oxidase method. Inorganic phosphorus consumption was determined photocolorimetrically, observing its disappearance from the incubation medium. The P:O ratio was calculated in mitochondria incubated at 30°C. Oxygen uptake associated with Na,K-ATPase activity in rat brain slices was estimated by the GUBITZ *et al.* method (10). In brain homogenates Na,K-ATPase and Mg ATPase activities were estimated from ATP hydrolysis (18). The Student's t-test was applied to evaluate the statistical significance of data.

Results

Oxygen and glucose uptake of rat brain slices are shown in table I: 1 mM trazodone decreased oxygen consumption of slices incubated in Krebs-Ringer phosphate medium containing 105 mM K^+

Table I. *Effect of trazodone on oxygen and glucose uptake by rat brain slices.* Nine slices were used at each concentration. The values are means ± S.E.M.

Drug [mM]	K^+ [mM]	Ca^{2+} [mM]	O_2 uptake (μl/100 mg wet tissue/h)	Glucose uptake (mg/100 mg wet tissue/h)
Control	5	2.5	148.9 ± 11.4	1.01 ± 0.17
	105	2.5	213.9 ± 11.3	1.26 ± 0.06
	5	0.0	206.9 ± 14.5	1.03 ± 0.14
1	5	2.5	130.6 ± 7.3	0.53 ± 0.15 (a)
	105	2.5	160.2 ± 12.9 (a)	0.98 ± 0.13 (a)
	5	0.0	147.6 ± 12.0 (a)	1.00 ± 0.09
0.1	5	2.5	151.8 ± 5.8	0.70 ± 0.15
	105	2.5	194.3 ± 10.7	1.19 ± 0.12
	5	0.0	184.1 ± 11.7	0.84 ± 0.11
0.01	5	2.5	176.5 ± 12.1	0.71 ± 0.90
	105	2.5	182.8 ± 16.5	1.31 ± 0.15
	5	0.0	199.5 ± 13.9	1.01 ± 0.08
0.001	5	2.5	161.4 ± 8.4	0.85 ± 0.12
	105	2.5	215.3 ± 9.3	1.23 ± 0.09
	5	0.0	189.1 ± 12.7	1.08 ± 0.11
0.0001	5	2.5	180.9 ± 9.7 (a)	0.84 ± 0.14
	105	2.5	221.7 ± 6.3	1.27 ± 0.17
	5	0.0	209.4 ± 14.2	0.88 ± 0.11

(a) $p < 0.05$

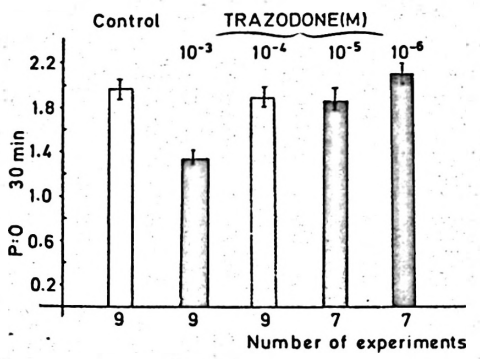

Fig. 1. *Effect of trazodone on the P:O ratio of brain mitochondria.*
The incubation contained 0.5 ml mitochondrial preparation with approximately 40 mg total protein, 0.2 ml 10 N NaOH in the center well. The gas phase was air and the temperature 30°C.

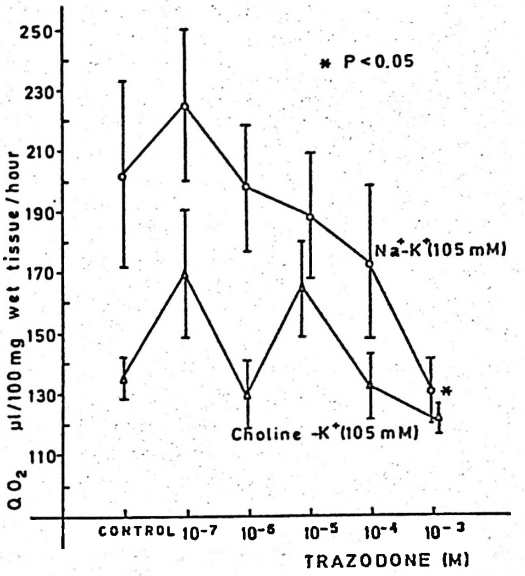

Fig. 2. *Effect of trazodone on oxygen consumption related to Na,K-ATPase activity in rat brain slices.*
Oxygen uptake of brain slices incubated in Krebs-Ringer phosphate 105 mM K^+ without Ca^{2+} (O). In this medium Na,K-ATPase activity of the membrane remains maximally stimulated. When they are incubated in the same medium but choline chloride replacing sodium chloride (Δ) the Na,K-ATPase activity is fully inhibited. Each point is the mean of at least 10 separate experiments.

or without Ca^{++}; 0.0001 mM trazodone increased oxygen uptake of slices incubated in Krebs-Ringer phosphate medium; 1 mM trazodone decreased glucose uptake of slices incubated in Krebs-Ringer phosphate medium containing 5 or 105 mM K^+. Trazodone produced no effect at concentrations 0.1, 0.01 and 0.001 mM.

Table II. *Effect of trazodone on oxygen uptake by rat brain homogenates incubated with substrates and cofactors.*
Nine experiments were performed for each concentration. The values are means ± S.E.M.

Drug [mM]	Oxygen uptake (µ/100 mg wet tissue/h)	%
Control	352.4 ± 24.1	
1	204.9 ± 27.4 (b)	— 42.0
0.1	351.3 ± 32.8	—
0.01	409.4 ± 13.3	—
0.001	423.8 ± 15.9 (a)	+ 20.3
0.0001	417.2 ± 9.6 (a)	+ 18.4

(a) $p < 0.05$; (b) $p < 0.01$.

Oxygen consumption of rat brain homogenates was decreased by 1 mM trazodone and increased by lower concentrations i.e. 0.001 and 0.0001 mM (table II).

Trazodone 1 mM decreased P:O ratio of rat brain mitochondria (fig. 1).

The oxygen consumption related to Na,K-ATPase activity of the membrane was decreased by 1 mM trazodone (fig. 2). Na,K-ATPase activity of membrane was reduced by 0.1 mM trazodone.

The dopamine (0.0001 and 0.001 mM) induced increase of oxygen consumption by rat brain slices was abolished by incubation with 0.0001 mM trazodone (figure 3).

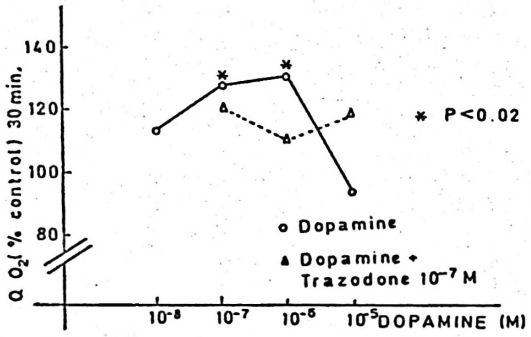

Fig. 3. *Effect of dopamine on rat brain slices incubated with trazodone.*
Oxygen uptake was determined manometrically at 37°C in rat brain slices incubated in Krebs-Ringer phosphate medium (pH 7.4). Dopamine was assayed either alone in the liquid phase of the experimental flasks or added from their side arm, when trazodone (0.0001 mM, final concentration) was previously placed in the liquid phase of the flasks. Results are mean of 9 experiments expressed as percentage of control values (x ± S.E.M. = = 102.4 ± 2.9 μl O_2/100 mg w.w.) at 30 min incubation plotted against molar concentration of dopamine.

Discussion

Results of oxygen and glucose uptake by brain slices, either used as controls (5 mM K^+) or stimulated (105 mM K^+, Ca^{++}-free medium) are in good accordance with previously published ones (4, 5, 16).

A concentration of 1 mM trazodone reduces QO_2 of rat brain homogenate incubated in sucrose pH 7.4 and uncouples oxidative phosphorylation, such as dibenzepine hydrochloride (16), maprotiline hydrochloride (8) and doxepin hydrochloride (5), although some «atypical» antidepressants like nomifensin maleate (9) and mianserin (4) are not uncouplers of oxidative phosphorylation.

Trazodone reduces QO_2 of rat brain slices incubated in Krebs-Ringer solution without calcium or when an excess of potassium is present in the medium, i.e. is when the Na,K-ATPase activity of the membrane is stimulated, which suggests some degree of inhibition of the enzyme. This result is confirmed by experiments with trazodone on ATPase activity of rat brain cortex homogenate. Na,K-ATPase activity was reduced by 0.1 mM trazodone. Moreover we have used higher concentrations of drug, in the range of those imipramine decrease Na,K-ATPase activity on guinea pig brain (2) and on ox brain (11).

Dopamine-induced increase of QO_2 of rat brain slices incubated in Krebs-Ringer phosphate was abolished by 0.0001 mM trazodone. This antagonism is similar to the effect of several other drugs described by our group: nomifensin maleate (9), clozapine (12), haloperidol (8), tiapride (17), pimozide (13), mianserin (4), doxepin (6) and clothiapine (5).

These results can be explained by a specific antagonism with dopamine as much as by a stabilizing effect on membrane (2, 11, 14).

Resumen

El trazodone (1 mM) reduce el consumo de oxígeno de homogeneizado de cerebro de rata, así como la actividad ATPasa-Na,K dependiente y el consumo de oxígeno asociado de cortes de cerebro y disminuye significativamente la relación P:O en mitocondrias de cerebro de rata. A 0,0001 mM anula el incremento del consumo de oxígeno inducido por dopamina en cortes de cerebro de rata.

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