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PRELIMINARY COMMUNICATION

EFFECT OF ZINC ON [^{3}H]-QNB DISPLACEMENT BY CHOLINERGIC AGONISTS AND ANTAGONISTS

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Aronstam et al.(1,2) have shown that zinc and other heavy metals or sulfhydryl reagents increase the affinity of [^{3}H]-QNB (quinuclidinyl benzilate) binding sites for cholinergic agonists in rat forebrain. These authors suggested that both heavy metals and N-ethylmaleimide (NEM) affect agonist binding by a common mechanism, possibly involving sulfhydryl groups. In this report, we demonstrate that zinc treatment of rat forebrain membranes enhances [^{3}H]-QNB displacement by cholinergic agonists but not cholinergic antagonists. Physiologically, zinc is believed to be important in maintaining neurotransmission and axonal transport (3,4).

MATERIALS AND METHODS

Atropine, oxotremorine, arecoline, pilocarpine, acetylcholine, physostigmine and zinc sulfate were purchased from Sigma (St. Louis, MO). Clozapine and thioridazine were obtained from Sandoz (East Hanover, NJ). Amitriptyline and benztropine were purchased from Merck, Sharp and Dohme (West Point, PA). Imipramine was obtained from Ciba-Geigy (Summit, NJ) and scopolamine was from Penick (Newark, NJ). [^{3}H]-L-Quinuclidinyl benzilate, 33.1 Ci/mmole, was purchased from New England Nuclear (Boston, MA). Zinc chloride was obtained from Mallinckrodt (Paris, KY).

Membranes were prepared from male Wistar rat brain tissue by homogenization at 0.009-0.010 cm clearance in 10 vol. of 50 mM sodium-potassium phosphate buffer, pH 7.4, or 50 mM Tris buffer, pH 7.4, containing 1 mM $ZnSO_4$ or $ZnCl_2$. The supernatant fraction of a 10-minute centrifugation at 1,000 g was centrifuged at 50,000 g for 1 hr. The pellets were resuspended and incubated in the buffer, in which they were homogenized. Tris-HCl buffer was used for the zinc-treatment due to the insolubility of high concentrations of zinc in phosphate buffer. A separate experiment showed that tris buffer alone had no effect on ^{3}H-QNB binding and preparation of forebrain membranes in either 1 mM $ZnCl_2$ or 1 mM $ZnSO_4$ resulted in identical effects.

The binding of [^{3}H]-QNB, a specific and potent muscarinic antagonist, was assayed according to Yamamura and Snyder (5) as modified by Aronstam et al.(2). A suspension

containing 0.1 mg of membrane protein, a 50 mM concentration of the appropriate pH 7.4 buffer and [^{3}H]-QNB (1 nM), with or without 10 μM atropine, was incubated for 40 min at 20°, filtered by suction through Whatman GF/B filters, and then rinsed with three 5-ml washes of appropriate pH 7.4 buffer. The filters were counted in Liquiscint counting solution (National Diagnostics) at 33% efficiency. Protein was determined by the Bio-Rad method (6).

Inhibitory constants (K_i) were calculated from the formula

$$K_i = \frac{IC_{50}}{1 + \frac{[L]}{K_D}}$$

as described by Cheng and Prusoff (7). The IC_{50} values were derived from log-probit analysis of competitive binding assays and the different K_D values for control and zinc-treated groups were determined by Scatchard analysis of equilibrium binding of [^{3}H]-QNB (Table 1).

RESULTS

Binding of 1 nM [^{3}H]-QNB to forebrain membranes subsequent to homogenization in 1 mM Zn^{2+} showed a slight decrease in specifically bound [^{3}H]-QNB compared to control (25%, N=11) with no change in nonspecific binding (in the presence of 10 μM atropine). This decrease agrees with that reported by Aronstam et al. (2), using 1 mM $ZnSO_4$. Three saturation experiments, employing 12 [^{3}H]-QNB concentrations (0.05 to 0.95 nM) in the presence and absence of 1 mM Zn^{2+}, showed a small increase in binding sites and a 3- to 4-fold decrease in [^{3}H]-QNB binding affinity in zinc-treated tissue. Hill coefficients of approximately one were observed in both control and treated preparations.

Table 1. Effect of zinc on the equilibrium binding parameters for [^{3}H]-QNB

	N	K_D (nM)	B_{max} (pmol/g protein)	n_H
Control	3	0.12 ± 0.03	1.09 ± 0.19	1.00 ± .004
Zinc-treated	3	0.44 ± 0.10*	1.35 ± 0.28	1.03 ± .020

*Different from control ($p<0.10$), using a two-tailed Student's paired t-test.

The ability of cholinergic agonists and antagonists to displace 1 nM [^{3}H]-QNB in control or zinc-treated forebrain membranes is shown in Table 2. Agonists showed an increased affinity for the [^{3}H]-QNB labeled receptor in the presence of zinc as demonstrated by shifts to the left of the binding isotherms and lower K_i values. Acetylcholine and carbachol, which showed very weak inhibition of binding in the absence of zinc, gave

complete inhibition curves in the presence of zinc. There was either no change or a slight increase in K_i values for antagonists. This suggests a small zinc-induced decrease in the affinity for some antagonists, which agrees with the equilibrium binding data.

Table 2. Displacement of 1 nM [^{3}H]-QNB in control and zinc-treated membranes

		K_i		
Compound	N	Control	Zinc-treated	Control/Zinc-treated
Acetylcholine	3	not determined*	1.06 ± 0.48 μM	>10.00
Methacholine	3	21.21 ± 3.03 μM	2.17 ± 0.63 μM	10.90
Carbachol	3	not determined*	2.73 ± 1.23 μM	> 3.90
Arecoline	3	5.51 ± 0.69 μM	1.80 ± 0.15 μM	3.10
Pilocarpine	3	5.80 ± 2.45 μM	1.72 ± 0.80 μM	3.40
Oxotremorine	4	0.29 ± 0.04 μM	0.19 ± 0.05 μM	1.50
Atropine	5	0.42 ± 0.05 nM	0.51 ± 0.03 nM	0.81
Benztropine	3	0.55 ± 0.03 nM	0.55 ± 0.12 nM	1.00
Scopolamine	3	0.34 ± 0.09 nM	0.95 ± 0.16 nM	0.36
Clozapine	3	6.08 ± 2.74 nM	9.91 ± 2.16 nM	0.61
Thioridazine	3	8.32 ± 2.33 nM	14.56 ± 4.27 nM	0.57
Amitriptyline	3	7.04 ± 2.64 nM	8.10 ± 0.38 nM	0.87
Imipramine	3	50.58 ± 3.34 nM	64.73 ±12.91 nM	0.78

*IC_{50}>100 μM

DISCUSSION

Preparation and incubation of rat forebrain in 1 mM Zn^{2+} enhanced [^{3}H]-QNB displacement by cholinergic agonists but not by cholinergic antagonists. The weaker agonists, arecoline, pilocarpine and carbachol,show more of a shift in the presence of 1 mM Zn^{2+} than does oxotremorine (see Table 2). It is interesting to note that the former cholinergic agonists also enhanced carbachol displacement of [^{3}H]-QNB from NEM-treated tissue, if present during NEM treatment (2), but oxotremorine was less effective.

Zinc ions play important roles in the formation of tubulin transport sheets and increasing the number of neurofilaments (8), and enhancing axonal transport *in vitro* (9). Another line of evidence suggests that zinc is important in the maintenance of neurotransmission, since abnormal decrements in responses to low-frequency stimulation of hippocampal mossy fibers occur in zinc-deficient rats (3). The results presented in this report describe a selective agonist-enhancing effect of zinc at the muscarinic receptor *in vitro*.

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REFERENCES

1. R. S. Aronstam, W. Hoss and L. G. Abood, Eur. J. Pharmac. 46, 279 (1977).
2. R. S. Aronstam, L. B. Abood and W. Hoss, Molec. Pharmac. 14, 575 (1978).
3. G. W.Hesse, Science 205, 1005 (1979).
4. C. C. Pfeiffer and E. R. Braverman, Biol. Psychiat. 17, 513 (1982).
5. H. I. Yamamura and S. H. Snyder, Proc. nat. Acad. Sci. U.S.A. 71, 1725 (1974).
6. M. Bradford, Analyt. Biochem. 64, 509 (1976).
7. Y. C. Cheng and W. H. Prusoff, Biochem. Pharmac. 22, 3099 (1973).
8. F. Gaskin, Y. Kress, C. Brosnan and M. Bornstein, Neuroscience 3, 1117 (1978).
9. A. Edstrom and H. Mattson, Brain Res. 86, 162 (1975).