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SHORT COMMUNICATIONS

The effect of desipramine on the noradrenaline stimulated Na–K ATPase of rabbit synaptic membranes

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Sodium, potassium-activated adenosine triphosphatase (Na–K ATPase, EC 3.6.1.3) is thought to be an enzymic representation of the sodium–potassium pump [1]. Several investigators have reported that Na–ATPase in brain tissue was stimulated by biogenic amines such as dopamine, noradrenaline and serotonin [2–6]. At present we do not know the physiological significance of the biogenic amine stimulation of synaptic membrane Na–K ATPase, although a few possibilities are discussed in connection with biogenic amine transport [5] and slow inhibitory postsynaptic potentials generated by biogenic amines [7]. This paper examines the effects of inhibitors of noradrenaline uptake on the noradrenaline stimulation of Na–K ATPase.

Albino male rabbits were stunned and decapitated. The brain was rapidly exposed and removed to ice-cold isotonic sucrose (0.32 M). The cerebral cortex was removed and homogenized in 10% w:v ice-cold isotonic sucrose, by six thrusts of a power-driven (25 mm diameter) Teflon pestle (clearance 0.18 mm). The speed of the motor was 900 r.p.m.

The homogenate was centrifuged at 1000 *g* for 20 min in a Sorvall Model SS1 centrifuge with an 8 × 50 ml angle head. The pellet was discarded and the supernatant centrifuged at 20,000 *g* for 30 min. The supernatant was discarded and the pellet was washed once and re-suspended in isotonic sucrose. The resultant suspension was centrifuged at 30,000 *g* for 30 min. The pellet was re-suspended in isotonic sucrose and laid onto a discontinuous density gradient (15 ml of 1.2 M sucrose, 10 ml of 0.8 M sucrose) in a 50 ml centrifuge tube and centrifuged at 30,000 *g* for 2 hr on an 8 × 50 ml angle head of the MSE SS 40 centrifuge. The synaptosomal fraction was collected at the 0.8/1.2 M sucrose interphase. The isotonicity was restored by slowly adding 4½ vol. of 0.16 M sucrose. The suspension was centrifuged at 30,000 *g* for 30 min and the pellet re-suspended in isotonic sucrose. Synaptic membranes were prepared by osmotically rupturing the synaptosomes using distilled water and centrifuging the resultant suspension at 50,000 *g* for 1 hr. Synaptic membranes which are rich in Na–K ATPase are pelleted by this procedure.

ATPase were assayed by determination of the rate of release of inorganic phosphate (Pi). Membranes were incubated at 37°, unless otherwise stated, in a 50 mM histidine HCl buffer, pH 7.4 (unless otherwise stated). Total ATPase activity was assayed in a medium containing 150 mM Na, 10 mM K, 3 mM Mg and 3 mM ATP. The Mg ATPase was assayed in a medium containing 3 mM Mg and 3 mM ATP. All data presented here was obtained using Boeheringer disodium ATP. The membranes were added to a final concentration of 100 μg/ml and the incubation was commenced by the addition of ATP.

The incubations were stopped by adding 4 ml of colour solution prepared by dissolving 10 g of ammonium molybdate and 10 g of Lubrol WX in 1 l of 0.9 M H_2SO_4. The colour was left to develop and was stable for 3 hr at room temperature. Noradrenaline (10^{-4} M) affects the estimation of Pi by this method. When possible this was overcome by using noradrenaline at concentrations not greater than 20 μM. However, if the concentration of noradrenaline greater then 20 μM were required, standard Pi graphs were prepared for each noradrenaline concentration used. The drugs also affected the estimation of Pi. Standard graphs were prepared for each drug.

Protein estimation was performed by the method of Lowry *et al.* [8].

Noradrenaline did not affect the degree of inhibition of Na–K ATPase by harmaline. Phentolamine 10 μM, an adrenoreceptor antagonist, and chlorpromazine 10 μM, which has both adreno and dopaminergic receptor antagonist properties, did not affect Na–K ATPase; however, both were potent inhibitors of noradrenaline induced stimulation of Na–K ATPase (Table 1). The drug concentration required to inhibit the noradrenaline stimulation of enzyme by 50 per cent was calculated from a plot of percentage inhibition of the stimulation of Na–K ATPase by 10^{-5} M noradrenaline against the logarithm of the drug concentration. Phentolamine was the most potent: IC_{50} values phentolamine, $2.0 \pm 0.9 \times 10^{-8}$ M, chlorpromazine, $3.5 \pm 0.6\ 10^{-7}$ M, desipramine, $1.2 \times 0.1 \times 10^{-5}$ M (mean ± S.D.). The antagonism of the noradrenaline response by phentolamine could be reversed by increasing the concentration of noradrenaline. However, desipramine was a non-competitive inhibitor of the noradrenaline stimulation of Na–K ATPase (Fig. 1).

The reuptake of noradrenaline into presynaptic terminals

Table 1. Effects of psychotropic drugs on Na–K ATPase of rabbit cerebral synaptic membranes

	Na–K ATPase μmoles Pi/mg/hr		Net increase* in Na–K ATPase
	[NA] = 0	[NA] = 10^{-5} M	
Control	18.7 ± 0.9	25.3 ± 1.45	6.7 ± 0.95
Phentolamine	19.1 ± 1.5	20.3 ± 1.4	1.1 ± 0.2‡
Chlorpromazine	18.9 ± 0.9	20.7 ± 1.6	1.8 ± 0.5‡
Desipramine	18.8 ± 1.4	22.3 ± 1.9	3.3 ± 0.6†
Imipramine	18.9 ± 1.1	23.7 ± 1.9	5.0 ± 0.8†

* Mean difference in Na–K AtPase activity ± S.D. produced by 10^{-5}M noradrenaline. Data obtained from 11 experiments.
† $P < 0.01$.
‡ $P < 0.001$.

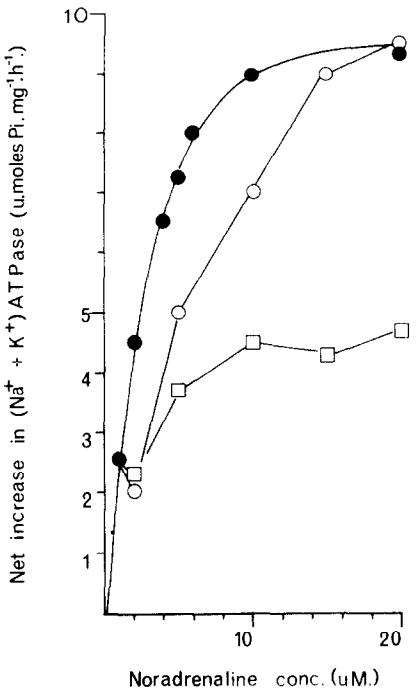

Fig. 1. The net increase in Na–K ATPase activity plotted as a function of the noradrenaline concentration, -●- control and in the presence of -○- phentolamine ($ID_{50} \simeq 10^{-7}$ M) or -□- desipramine ($ID_{50} = 10^{-5}$ M).

is ouabain sensitive, suggesting that the presynaptic membrane Na–K ATPase is involved in the amine uptake mechanism. The possibilities are that Na–K ATPase may generate the correct ionic environment for the operation of the amine pump of the enzyme may have a more direct role in the transport of noradrenaline. The former suggestion is supported by the requirement of the amine pump for a high external $[Na^+]$. However, the noradrenaline uptake system of cerebral nerve endings is unique among amino acid and sugar transport mechanisms found in neural tissue in that the affinity of the carrier is not affected by $[Na^+]_0$ [9] and further, an inward directed sodium gradient is not necessary for the re-uptake of noradrenaline [10].

The amine may have an allosteric interaction with the enzyme to increase its rate of working, thus providing more energy for the transport mechanism. However, there was no evidence from the studies on the effect of noradrenaline concentration on the increase in Na–K ATPase activity to suggest that there was such an allosteric interaction. It is not inconceivable that the amine could compete with one of the monovalent ions for occupancy of a site on the enzyme; however, the optimum conditions for noradrenaline stimulation of the Na–K ATPase were the same as the optimum conditions for Na–K ATPase, and further increasing the $[K^+]_0$ or $[Na^+]_0$ did not decrease the effects of noradrenaline on the Na–K ATPase activity. Phentolamine, a competitive inhibitor of the amine activation of Na–K ATPase (ID_{50} 10^{-8} M) had no effect on the activity of the Na–K ATPase at drug concentrations in excess of 10^{-5} M. The possibility of the existence of a specific species of ATPase requiring noradrenaline for its activity is invalidated by the observation that the increase in Na–K ATPase in the presence of noradrenaline does not show affinity saturation kinetics with respect to [ATP].

The order of potency of drugs inhibiting the uptake of noradrenaline by synaptosomes is desipramine > imipramine > chlorpromazine [11]. Phentolamine is a very weak inhibitor of NA uptake. In this study, the order of potency of drugs antagonising the noradrenaline activation of Na–K ATPase was quite the reverse; phentolamine > chlorpromazine > desipramine. Indeed, desipramine is 10^3 × more potent as a competitive inhibitor of noradrenaline uptake than as a non-competitive antagonist of noradrenaline stimulation of Na–K ATPase. These results indicate that the characteristic noradrenaline stimulation of Na–K ATPase is not identical with the characteristics of the presynaptic noradrenaline uptake system.

To summarise, the activation of Na–K ATPase by noradrenaline was antagonised by phentolamine > chlorpromazine > desipramine. Phentolamine was a competitive antagonist and desipramine was a non-competitive antagonist of the noradrenaline effect on the enzyme.

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