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A KINETIC ANALYSIS OF DRUGS THAT INHIBIT THE ADRENERGIC NEURONAL MEMBRANE AMINE PUMP*

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Abstract—Kinetic analysis of uptake of *levo*-metaraminol by rabbit heart slices reveals that cocaine, desipramine, chlorpromazine, and bretylium competitively inhibit amine uptake by the adrenergic neuronal membrane amine pump. Ouabain also inhibits, but by a noncompetitive mechanism. It is concluded that the membrane amine pump is linked energetically to the cardiac glycoside sensitive Na^+-K^+-dependent ATPase.

RECENT studies based on histological and biochemical evidence have demonstrated the existence of two amine concentrating mechanisms in the adrenergic neurone.[1–3] One operates at the level of the neuronal membrane to facilitate uptake of amines from the extracellular fluid while the other operates intraneuronally at the level of the amine storage granules. The latter mechanism is blocked specifically by reserpine, tetrabenazine, and low concentrations of guanethidine, whereas the membrane mechanism is blocked specifically by cocaine, bretylium, ouabain, and imipramine and its congeners.[3] The membrane mechanism is an energy-requiring system, which can be demonstrated to obey Michaelis–Menten kinetics, and thus may be considered to be an amine pump.

The present study is concerned with the kinetics of amine uptake by the membrane pump and the mechanism of drugs interfering with its action. The amine used as a pump 'substrate' in these studies is *levo*-metaraminol, a compound which is not metabolized by either monoamine oxidase or catechol-*O*-methyl transferase. As described previously, an agent interfering with the uptake of this amine by heart slices does so by inhibition of the membrane amine pump.[3] It is demonstrated in this study that cocaine, desipramine, chlorpromazine, and bretylium act as competitive inhibitors of the membrane amine pump, whereas ouabain acts as a noncompetitive inhibitor, suggesting that the amine pump is linked energetically with glycoside sensitive Na^+-K^+-dependent ATPase.

METHODS AND MATERIALS

Adult albino rabbits were killed by air embolism. Hearts were excised and ventricle slices were prepared and incubated in Krebs–Ringer phosphate buffer, pH 7·4, under an atmosphere of O_2 at 37°, as described previously.[3] *Levo*-metaraminol (*l*MA) was added after a 15-min preincubation period. Drugs inhibiting *l*MA uptake were added at the beginning of the preincubation period. Incubation with *l*MA was for

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30 min, uptake being linear over this time. *l*MA levels were measured in the slice fluorometrically as described elsewhere.[4] The accumulation of *l*MA by the slice was calculated as net uptake (concentration/ml slice water minus medium concentration). The concentration of inhibitor drug required to inhibit *l*MA uptake by 50 per cent was estimated by plotting the per cent inhibition of net uptake against log concentration of inhibitor drug. The resulting ED_{50} for each drug was used in the kinetics study. Lineweaver–Burk plots were constructed and K_m was estimated by the method of Dixon.[5]

RESULTS AND DISCUSSION

Table 1 shows the ED_{50} of various drugs inhibiting the membrane amine pump. Of the various inhibitors, desipramine was the most active, the ED_{50} being about 3×10^{-8} M. The ED_{50} concentrations were used in the following experiments.

TABLE 1. CONCENTRATIONS OF DRUGS INHIBITING *l*-METARAMINOL UPTAKE BY HEART SLICES BY 50 PER CENT*

Drug	ED_{50}
Desipramine	3×10^{-8} M
Chlorpromazine	2×10^{-7} M
Cocaine	2×10^{-6} M
Ouabain	3×10^{-6} M
Bretylium	2×10^{-5} M

* The effect of various concentrations of each drug in inhibiting *l*MA uptake (medium concn. = 0·1 μg/ml) was plotted on semi-log paper and ED_{50} determined graphically.

Fig. 1 shows Lineweaver–Burk plots of *l*MA uptake with no inhibitor added and in the presence of cocaine or ouabain. It may be noted that the K_m of the uninhibited experiment is about 0·2 μg/ml (10^{-6} M). In the presence of cocaine, a marked inhibition of *l*MA uptake can be seen, but the ordinate intercept is unchanged, indicating competitive inhibition. Similar kinetic studies with desipramine, chlorpromazine, and bretylium as inhibitors showed that these compounds also did not alter V_{max} of *l*MA uptake. All of these agents thus must be considered competitive inhibitors. Ouabain, on the other hand, significantly decreased V_{max} without altering K_m, indicating a noncompetitive type of inhibition.

It is perhaps not surprising that a number of drugs inhibit amine uptake by competing for the membrane amine carrier, since it has been demonstrated that the membrane amine pump is relatively nonspecific in its choice of substrates.[3] The nonspecificity of this pump is to be contrasted with the considerable specificity of the intracellular (granular) concentrating mechanism.[6, 7]

Of the various inhibitors tested in this study, only ouabain exhibited a more complex action. The noncompetitive nature of its action suggests an effective decrease in the number of carrier sites or in the energy supplied to the amine pump. Since

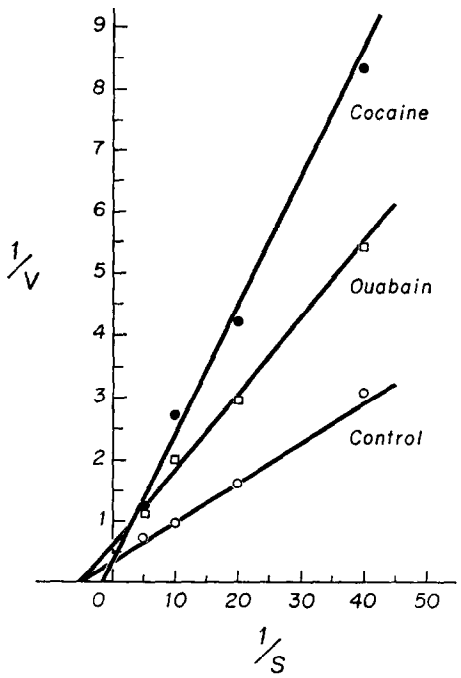

FIG. 1. Lineweaver-Burk plot of *l*MA accumulation in rabbit heart slices and inhibition by cocaine 2×10^{-6} M) and ouabain (3×10^{-6} M). Velocity (V) is taken to be net uptake of *l*MA by slice during 30-min incubation. Substrate concentration (S) is μg *l*MA/ml medium. Ordinate intercepts of control vs. cocaine-treated are identical, while intercepts of control v. ouabain-treated differ significantly ($P < 0{\cdot}05$). Curves shown were calculated by linear regression analysis. Each point represents the mean of 6 to 8 experiments. Correlation coefficients are: control, 0·98; ouabain, 0·96; cocaine, 0·95. In each case the linear regression model is appropriate for the data at the 0·0005 level of significance.

the known biochemical action of ouabain is one of inhibition of the Na^+-K^+-dependent ATPase, and since it has been demonstrated that both amine uptake and Na^+-K^+-dependent ATPase activity are inhibited by a low Na^+ concentration,[8, 9] it would seem that ouabain action on amine uptake is secondary to its effects on ATPase. Consistent with this is our finding (unpublished) that the action of various digitalis-related compounds on *l*MA uptake is proportional to cardiotoxicity which in turn is proportional to Na^+-K^+-dependent ATPase inhibition.[10] Thus cassaine has only one-tenth the action of ouabain on *l*MA uptake by heart slices and is about one-tenth as toxic as ouabain in the cat.[11, 12] Dihydrostrophanthidinic acid, which is not cardiotoxic,[13] does not inhibit *l*MA uptake even at high concentration (10^{-4}M). Finally, in the rabbit the cardiotoxic activity of ouabain and its inhibitory action on amine uptake are high, whereas ouabain is almost devoid of both these actions in the rat and the ATPase of the latter species is insensitive to ouabain.[10]

It is not likely that the amine is simply substituting for inorganic cations in Na^+-K^+ movements. If this were so, one would expect amine accumulation in muscle cells, Na^+-K^+-dependent ATPase being present in most mammalian tissues, whereas it is known that *l*MA accumulation occurs only in adrenergic neurones.[3]

A more likely explanation of the ouabain effect is that the glycoside interferes with energy transfer required in a linked Na^+-K^+-dependent ATPase–amine pump mechanism. Such a linked mechanism has been suggested for amino acid transport in striated muscle.[14]

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