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INTERACTION OF RESERPINE AND OUABAIN ON AMINE CONCENTRATING MECHANISMS IN THE ADRENERGIC NEURONE*

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Abstract—Reserpine does not alter the accumulation of *l*- or *d*-metaraminol in the adrenergic neurones of rabbit heart slices. Ouabain and desipramine decrease uptake by inhibition of the neuronal membrane amine pump, but the preference of the concentrating mechanism for the *levo* form is retained. In the reserpinized heart slice, ouabain action on accumulation of *l*-metaraminol is greatly enhanced, but a similar potentiation of ouabain action on *d*-metaraminol does not occur. Reserpine does not enhance the inhibitory action of desipramine, nor does tetrabenazine duplicate the ouabain-enhancing effect of reserpine on *l*-metaraminol accumulation. It is suggested that reserpine has a separate effect over and above a tetrabenazine-like action on amine storage granules, and, perhaps by creating a local ionic imbalance in the cell, allows ouabain to exert a greater effect on a local Na^+-K^+-ATPase–amine pump linked mechanism which normally allows the *levo* form of catecholamines to be accumulated more rapidly than the *dextro* form.

STUDIES on the mechanism of amine accumulation by the adrenergic neurone have revealed the existence of two separate amine concentrating mechanisms, one operating as an amine pump at the neurone membrane, the other operating intracellularly at the level of amine storage granules.[1, 2] The granular mechanism is blocked specifically by reserpine and tetrabenazine, whereas the membrane pump is blocked by cocaine, desipramine, and congeners, chlorpromazine, bretylium, and ouabain. Recently, kinetic studies on rabbit heart slices have revealed that, of the drugs inhibiting the membrane pump, ouabain acts by a noncompetitive mechanism, while the others act as competitive inhibitors.[3] Evidence was presented in support of a suggestion that ouabain acts indirectly by inhibition of a Na^+-K^+-dependent ATPase linked energetically to the membrane amine pump. The latter studies were carried out with the norepinephrine analog, metaraminol, as a substrate for the amine pump. This amine is not metabolized by monoamine oxidase or catechol-*O*-methyl transferase, and thus its rate of accumulation is a direct measure of amine transport, akin to the use of aminoisobutyric acid as a nonmetabolizable amino acid in studies on amino acid transport.

While both the *levo* (*l*MA) and *dextro* (*d*MA) forms of metaraminol are substrates of the membrane amine pump and are accumulated readily, only *l*MA is stored in the intraneuronal granules and only *l*MA displaces norepinephrine from this site.[4] In

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this regard it should be noted that the β hydroxyl group of *l*MA has the same absolute configuration as that of *l*-norepinephrine.[5]

The present studies demonstrate that reserpine, which does not alter accumulation of *l*MA or *d*MA by the heart slice, does, however, markedly potentiate the inhibitory action of ouabain on accumulation of *l*MA but not *d*MA. Reserpine does not enhance the effect of desipramine on *l*MA uptake, nor does tetrabenazine duplicate the effect of reserpine in potentiating ouabain action. The results suggest that reserpine has a separate action of unmasking a ouabain-sensitive site which is stereospecific for the *levo* form of catecholamines or may create a local ionic imbalance such that local effects of ouabain on Na^+-K^+-ATPase are enhanced.

METHODS AND MATERIALS

Rabbits were killed by air embolism, hearts removed, and ventricle slices prepared and incubated in buffer in a metabolic shaker as described previously.[2] *Levo*-metaraminol (*l*MA) or *dextro*-metaraminol (*d*MA) were added after a 15-min preincubation period to make a medium concentration of 0·1 μg/ml. Incubation period was 30 min. Drugs inhibiting amine uptake were added at the beginning of the preincubation period, with the exception of reserpine, which was administered (5 mg/kg, i.v.) to rabbits 18 hr before killing. Metaraminol levels were measured fluorometrically as described elsewhere.[6] The accumulation of metaraminol by the slice was calculated as "net uptake" (concentration per ml slice water minus medium concentration) as described previously.[2] Measurement of uptake after 30 min of incubation does not reflect steady state concentrations, since metaraminol accumulation under the above conditions normally does not reach saturation levels until about 2 hr of incubation.

RESULTS

As previously observed, *l*MA accumulates greatly in the heart slice.[2] Reserpine pretreatment did not alter the extent of uptake, but ouabain, 5×10^{-6}M, inhibited accumulation by about 50 per cent. Reserpine treatment prior to the addition of ouabain resulted in a marked potentiation of the inhibitory action of the glycoside (Table 1). Treatment of the slices with tetrabenazine at a concentration (10^{-5}M) known to inhibit the granular amine storage mechanism, like reserpine, did not alter accumulation of *l*MA.[2] Unlike reserpine, however, tetrabenazine did not potentiate the inhibitory effect of ouabain (Table 1).

Desipramine ($3{\cdot}3 \times 10^{-8}$M) also inhibited *l*MA accumulation by about one-half, but its effect was not altered by reserpine pretreatment (Table 2).

Studies with *d*MA revealed that, although ouabain inhibited uptake of this amine to about the same extent as *l*MA, reserpinization did not enhance the inhibitory effect of the glycoside on accumulation of the *dextro* isomer (Table 3).

Although the heart slice normally accumulates *l*MA to a greater extent than *d*MA, a preference retained even in the presence of ouabain, reserpinization allowed ouabain to abolish the stereospecificity of accumulation (Tables 1 and 3).

DISCUSSION

Previous studies have demonstrated the inhibitory effect of ouabain and desipramine on amine accumulation by the adrenergic neurone, and it has been concluded that these agents act at the level of the neuronal membrane amine pump. Recent kinetic

studies have revealed that desipramine and cocaine act as competitive inhibitors of the amine pump, while ouabain acts noncompetitively. Evidence was put forth to suggest that ouabain acts by inhibition of a linked Na^+-K^+-dependent ATPase–amine pump mechanism.[3]

TABLE 1. EFFECT OF OUABAIN ON *l*MA ACCUMULATION BY CONTROL, RESERPINIZED, OR TETRABENAZINE-TREATED RABBIT HEART SLICES*

Treatment	Net uptake of *l*MA (μg/ml ± S.E.)
None	0·70 ± 0·02
Reserpine	0·70 ± 0·02
Ouabain (5×10^{-6}M)	0·34 ± 0·01
Reserpine + ouabain	0·19 ± 0·01
Tetrabenazine (10^{-5}M)	0·75 ± 0·02
Tetrabenazine + ouabain	0·31 ± 0·02

* Experiments were carried out as described under Methods. Each figure represents results obtained from 6–12 experiments. Reserpine or tetrabenazine alone had no significant effect on accumulation. Enhancement of ouabain effect by reserpine treatment was highly significant ($P < 0·01$), whereas tetrabenazine had no effect on ouabain action.

TABLE 2. EFFECT OF DESIPRAMINE ON *l*MA ACCUMULATION BY CONTROL OR RESERPINIZED RABBIT HEART SLICES*

Treatment	Net uptake of *l*MA (μg/ml ± S.E.)
None	0·70 ± 0·01
Reserpine	0·70 ± 0·01
Desipramine ($3·3 \times 10^{-8}$M)	0·40 ± 0·02
Reserpine + desipramine	0·40 ± 0·01

* Experiments were carried out as described under Methods. Each figure represents results obtained from 6–12 experiments. Reserpine had no significant effect on *l*MA accumulation by either control or desipramine-treated slices.

TABLE 3. EFFECT OF OUABAIN ON *d*MA ACCUMULATION BY CONTROL OR RESERPINIZED RABBIT HEART SLICES*

Treatment	Net uptake of *d*MA (μg/ml ± S.E.)
None	0·43 ± 0·02
Reserpine	0·45 ± 0·02
Ouabain (5×10^{-6}M)	0·23 ± 0·02
Reserpine + ouabain	0·23 ± 0·02

* Experiments were carried out as described under Methods. Each figure represents results obtained from 6–12 experiments. Reserpine had no significant effect on *d*MA accumulation by either control or ouabain-treated slices.

Although reserpine or tetrabenazine alone are without effect on the membrane pump, their actions being confined to the intracellular (granular) storage mechanism, the present studies show that reserpine markedly potentiates the inhibitory action of ouabain on accumulation of *l*MA but not of *d*MA. The stereochemical preference of the uptake mechanism for the *levo* isomer is abolished in the presence of reserpine and ouabain.

Previous investigation has shown that *l*MA, but not *d*MA, is persistently stored in norepinephrine storage granules where it displaces endogenous catecholamines.[4] Thus, it might seem that one explanation of reserpine enhancement of ouabain would be that reserpine, blocking the optically selective granular storage mechanism, would decrease the degree of intracellular binding of *l*MA but not of *d*MA, thus allowing an agent (ouabain) acting on the membrane pump to exert an apparently greater effect on *l*MA due to increased rate of washout of *l*MA but not of *d*MA. Several points argue against this, however. First, reserpine alone does not affect accumulation of either *l*MA or *d*MA by the slice. Second, reserpine does not enhance the action on *l*MA of another agent, desipramine, which acts on the membrane pump. Third, tetrabenazine, which has a reserpine-like effect on the granular storage meachnism, does not potentiate the effect of ouabain on *l*MA accumulation.

It would seem, then, that reserpine has a second action in this system in addition to blocking the granular storage mechanism. In simplest descriptive terms, reserpine seems to allow an action of ouabain on an amine concentrating mechanism which is specific for the *levo* form, but this statement begs the question of the mechanism involved. The lack of effect of reserpine on the action of desipramine on *l*MA accumulation and the lack of effect of reserpine on the action of ouabain on *d*MA accumulation bring up the possibility that a separate ouabain-sensitive site is specifically responsible for transport or storage of the *levo* form over and above that of the *dextro* form. This separate ouabain-sensitive site might be manifested at the membrane level or intracellularly, but independent of the tetrabenazine and reserpine-sensitive granule storage mechanism.

Since the major biochemical action of ouabain is that of inhibition of the Na^+-K^+-ATPase, it may be that reserpine, perhaps by causing a local ionic imbalance at a specific site, may allow a local action of ouabain to be enhanced. This concept would fit with recent work showing that the inhibitory action of ouabain on an isolated cardiac Na^+-K^+-ATPase is highly dependent on the Na^+/K^+ ratio in the medium.[7]

It is interest that recent work has uncovered an action of reserpine in potentiating the toxic effect of *k*-strophanthin.[8, 9] These findings again bring up the possibility of reserpine enhancement of local glycoside actions on a Na^+-K^+-ATPase.

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