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DUAL ACTIONS OF GUANETHIDINE ON AMINE UPTAKE MECHANISMS IN ADRENERGIC NEURONS*

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Abstract—By using a technique which allows the differentiation *in vitro* between amine uptake mechanisms in adrenergic neurons, evidence is presented that guanethidine at 10^{-4} M inhibits both a membrane amine pump and an intracellular amine-concentrating mechanism. At a low concentration of guanethidine (5×10^{-6} M) the intracellular mechanism is preferentially inhibited. The action of guanethidine on the membrane pump is readily removed by washing. It is concluded that at clinical doses, guanethidine acts only on the intracellular mechanism, thus leading to depletion of norepinephrine.

A PREVIOUS study[1] described evidence obtained *in vitro* supporting the existence of two amine-concentrating mechanisms in adrenergic neurons[2, 3] as well as a convenient method for differentiation *in vitro* between the two mechanisms, thus allowing the elucidation of the site of action of drugs inhibiting amine uptake. The first mechanism, a neuronal membrane amine pump, is relatively nonspecific in its substrate requirements and is inhibited by cocaine, imipramine, and guanethidine, but not by reserpine or tetrabenazine. The second mechanism, an intracellular concentrating mechanism, presumably located at the cytoplasmic granule level, is much more specific in its substrate requirements and is inhibited by reserpine and tetrabenazine, but not by cocaine or imipramine. Amines taken into the cell by the membrane pump will, if monoamine oxidase (MAO) substrates, be destroyed unless sequestered by the intracellular mechanism. Thus, for example, the accumulation of *m*-octopamine, a MAO substrate, by rabbit heart slices is blocked by reserpine or tetrabenazine, but these drugs have no effect on the accumulation of metaraminol (MA) or α-methyl-*m*-tyramine, compounds which are not MAO substrates.[1] Cocaine or imipramine, on the other hand, inhibits the accumulation of MA and α-methyl-*m*-tyramine as well as that of *m*-octopamine.[1] That these mechanisms are functions of the adrenergic neuron is evident, as no uptake occurs in heart slices from immunosympathectomized animals with degenerated adrenergic neurons.[1]

As mentioned above, guanethidine was found to inhibit the membrane pump, but as guanethidine is a norepinephrine (NE)-depleting drug, it presumably also acts on the intracellular concentrating mechanism. Such a duality of guanethidine action was proposed by Lindmar and Muscholl[3] on the basis of measurement of NE taken up and retained by the perfused rat heart. The present paper demonstrates that the action of

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guanethidine on the membrane pump may be differentiated from a second effect—inhibition of the intracellular storage mechanism—which requires a much lower drug concentration. The findings thus provide further evidence for the existence of two amine-concentrating mechanisms and demonstrate a dual action of guanethidine.

METHODS

Adult albino rabbits were killed by air embolism. Hearts were excised, ventricle slices prepared and incubated in Krebs-Ringer phosphate buffer (pH 7·4) at 37°, and gassed with O_2 as described in a previous paper.[1] *l*-Metaraminol (MA) (0·1 or 0·025 μg/ml) or *l*-*m*-octopamine (0·1 μg/ml) was added after preincubation as indicated in Results. MA and *m*-octopamine were measured fluorometrically by methods described elsewhere.[4] Guanethidine sulfate was added at the beginning of the preincubation period. Comparison of amine uptake in guanethidine-treated versus control slices allowed calculation of inhibition of net amine uptake.

In other experiments, guanethidine sulfate was administered intraperitoneally to rabbits which were killed 1 hr later; heart slices were then prepared.

RESULTS

As noted previously,[1] rabbit heart slices accumulate both MA and *m*-octopamine. At equal substrate concentrations, about four times more MA than *m*-octopamine was accumulated. Therefore, to study effects of guanethidine on uptake of these amines, two substrate concentrations of MA were chosen for some experiments, one (0·1 μg/ml) equal to the substrate concentration of *m*-octopamine, and the other (0·025 μg/ml) which resulted in MA accumulation about equal to that of *m*-octopamine at the higher substrate concentration.

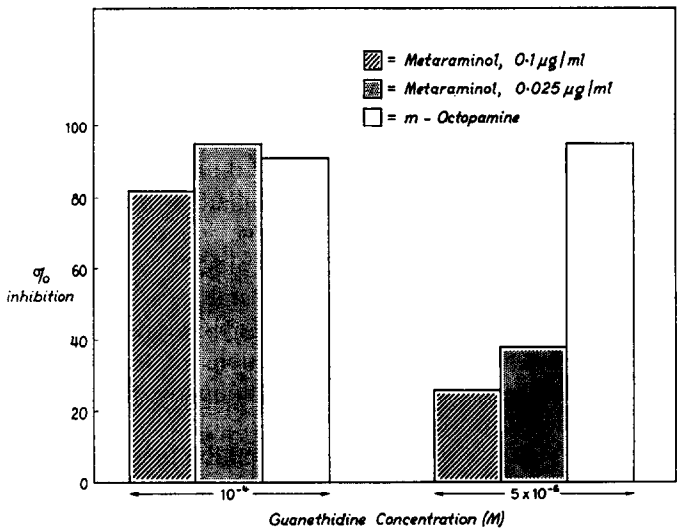

FIG. 1. Inhibition by guanethidine of accumulation of *l*-metaraminol or *l*-*m*-octopamine in rabbit heart slices. Slices were preincubated 15 min with 10^{-4} M or 60 min with 5×10^{-6} M guanethidine. Net uptake ±S.E. by control slices was: MA (0·1 μg/ml), 0·83 ± 0·06 μg/ml; MA (0·025 μg/ml), 0·27 ± 0·02 μg/ml; *m*-octopamine (0·1 μg/ml), 0·24 ± 0·01 μg/ml. Incubation period was 60 min. Each bar shows mean of 6–8 experiments.

Preincubation for 15 min with guanethidine (10^{-4} M) caused almost complete inhibition of uptake of both amines (Fig. 1). Preincubation for 15 min with 5×10^{-6} M guanethidine resulted in partial inhibition of *m*-octopamine uptake, but a much greater inhibition resulted when the preincubation was prolonged to 60 min (Fig. 2).

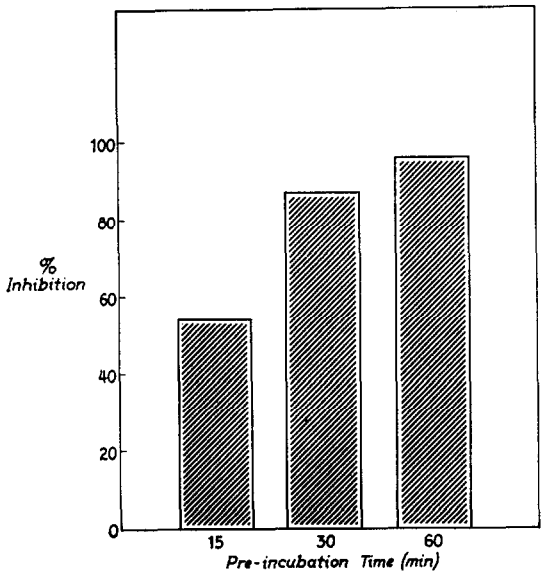

FIG. 2. Effect of preincubation time with guanethidine on accumulation of *m*-octopamine by rabbit heart slices. Slices were preincubated with guanethidine, 5×10^{-6} M, for the times indicated before addition of *m*-octopamine, 0·1 μg/ml, to the medium. Incubation period was 60 min. Each bar shows mean of 6–8 experiments.

A comparison of the effect of this lower guanethidine concentration on MA and *m*-octopamine accumulation showed that while *m*-octopamine accumulation was still maximally inhibited, there was relatively little inhibition of MA uptake (Fig. 1). Preincubation of control slices for 15 or 60 min showed that preincubation time per se did not affect amine uptake.

In other experiments, slices were preincubated for 15 min with 10^{-4} M guanethidine, but were then washed for 10 min with fresh buffer before being placed in buffer with MA or *m*-octopamine. As shown in Fig. 3, washing the slice virtually eliminated the inhibitory effect of guanethidine on MA uptake, but only partially decreased the inhibitory action of the drug on accumulation of *m*-octopamine.

When heart slices were prepared in the usual way from rabbits receiving guanethidine sulfate (20 mg/kg, i.p.) 1 hr before killing, there was little inhibition of MA uptake, but accumulation of *m*-octopamine was greatly inhibited (Table 1).

DISCUSSION

The results show clearly that whereas a high concentration of guanethidine (10^{-4} M) blocks the membrane pump, as is evidenced by the blockade of MA as well as *m*-octopamine uptake, incubation with a low drug concentration (5×10^{-6} M) inhibited the intracellular mechanism much more than the membrane pump, as is shown by the

preferential inhibition of *m*-octopamine accumulation. The dual action of guanethidine, proposed by Lindmar and Muscholl,[3] is thus apparent, and it is also clear that the drug effect on the intracellular mechanism is predominant, since it is manifested at concentrations so low as to have little effect on the membrane pump. Thus the action

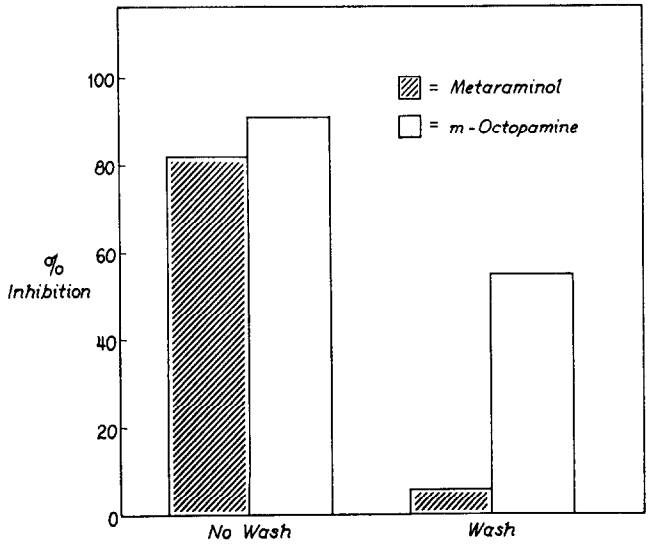

FIG. 3. Effect of washing slices after exposure to guanethidine. Rabbit heart slices were preincubated for 15 min with guanethidine (10^{-4} M). Slices were then transferred to a beaker containing 6 ml buffer and incubated 10 min before being transferred to still other beakers containing metaraminol or *m*-octopamine, 0·1 μg/ml. Incubation period was 60 min. Each bar shows mean of 6–8 experiments.

TABLE 1. ACCUMULATION OF METARAMINOL OR *m*-OCTOPAMINE BY HEART SLICES TAKEN FROM GUANETHIDINE-TREATED RABBITS

Treatment	Net uptake (μg/ml ± S.E.)	
	Metaraminol	*m*-Octopamine
None	0·83 ± 0·06	0·24 ± 0·01
Guanethidine	0·77 ± 0·07 (9% inhibition)	0·04 ± 0·01 (83% inhibition)

Normal rabbits or those pretreated 1 hr with 20 mg guanethidine sulfate/kg (i.p.) were killed and heart slices prepared and incubated with *l*-metaraminol or *l*-*m*-octopamine (0·1 μg/ml). Net uptake denotes total accumulation/ml slice water minus concentration in medium. Each figure shows mean of 6 to 8 experiments.

of guanethidine at 10^{-6} M to inhibit NE uptake by heart slices, as observed by Dengler,[5] must be due almost entirely to inhibition of the intracellular mechanism. This same conclusion is also supported by the finding that heart slices taken from rabbits treated with guanethidine show little *m*-octopamine accumulation but considerable MA uptake. It is possible, however, that the membrane pump was inhibited

in vivo after this large dose and that the subsequently removed slices lost sufficient drug to the medium that the resulting concentration was too low to affect the membrane pump. That this may be the case is indicated by the finding that inhibition at the membrane by a high concentration of guanethidine is readily reversed by washing the slices (Fig. 3). In clinical doses, however, it seems likely that only a reserpine-like effect on the intracellular NE storage mechanism is operant and that, in man, a cocaine-like effect of guanethidine on the membrane pump is not realized.

Schanker and Morrison[6] have shown that upon incubation of heart slices with low concentrations of guanethidine, uptake of the drug occurs slowly, reaching maximal levels 2 hr after incubation with 2 μg/ml (10^{-5} M). A reflection of this slow penetration of guanethidine is evident in the prolonged preincubation required for a low concentration of guanethidine to inhibit the intracellular mechanism maximally (Fig. 2).

The results reported not only demonstrate the dual action of guanethidine but, more importantly, provide new evidence supporting the existence of two amine-concentrating mechanisms.

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