Abstract—The uptake of guanethidine \(3.3 \times 10^{-5} \text{M}\), debrisoquin \(8.4 \times 10^{-4} \text{M}\), bretylium \(5.0 \times 10^{-4} \text{M}\) and xylocholine \(1.0 \times 10^{-3} \text{M}\) by adrenergic neuron was studied indirectly by testing the ability of noradrenaline (NA) regarding low temp. or sodium-deprivation to prevent or reverse adrenergic neuron blocking action in the indirectly stimulated guinea-pig hypogastric nerve vas deferens preparations. NA \(3.0 \times 10^{-6} \text{M}\) completely prevented the neuron blocking action of all the four blockers but reversed only that of bretylium. Exposure of preparations to a low temp. \(0^\circ\text{C}\) prevented the neuron blocking action of all the four blockers but did not reverse that of any one blocker. The adrenergic neuron blocking action of guanethidine was partially prevented but was not reversed by sodium-deprivation. Sodium-deprivation neither prevented nor reversed the neuron blocking action of the other three blockers. It is concluded that guanethidine shares the uptake mechanism of NA for transport across the neuron while debrisoquin, bretylium and xylocholine appear to be transported differently.

The uptake of noradrenaline (NA) by the adrenergic neuron is energy dependent (1, 2, 3) and sodium dependent (4, 5, 6, 7, 8, 9). Guanethidine utilises this NA uptake mechanism for transport across the membrane of neurons (10, 11) and of human platelets (12) which can serve as convenient model for the neuron (13). Support for this common transport mechanism for NA and guanethidine is derived from observations of the ability of NA to block the uptake of guanethidine, and the failure of uptake of guanethidine in sodium-deficient medium and under conditions of reduced energy supply as at low temp. (12). Gulati and Jaykar (14) showed that in the Finkleman preparation (15), the neuron blocking action of guanethidine was prevented by NA, sodium-deprivation and at low temp. and concluded that guanethidine shares the uptake mechanism for NA. Since the neuron blocking action of a neuron blocker is consequential upon the uptake by the neuron, the prevention of neuron block by various procedures and drugs affecting uptake of NA should provide information about the uptake of a neuron blocker. Thus the present communication is concerned with the investigation of the influence of NA, low temp. and sodium-deprivation in modifying the adrenergic neuron blocking action on Hukovic preparation (16) of guanethidine, xylocholine, bretylium and debrisoquin. In addition the mechanisms of the uptake of these neuron blockers into the adrenergic neurons are discussed.
MATERIALS AND METHODS

Male guinea pigs (300-450 g) were sacrificed by a blow on the head followed by incising the vessels in the neck. Both the vasa deferentia with intact hypogastric nerves were dissected out and set up as paired preparations (control and test) in isolated organ baths of 35 ml capacity in the manner described by Hukovic (16). The preparations were allowed to stabilize at 35°C for 30 min in Hukovic solution (16) which was continuously gassed with 95% oxygen and 5% carbon dioxide. The hypogastric nerve was stimulated supramaximally with square wave pulses of 0.5 msec duration every 5 min for 5 sec at frequencies of 3, 5, 7, 10, 20 and 60 Hz in that order or in a reverse order. The responses of vas deferens were recorded on a smoked paper (magnification 5X) with an isotonic lever under a load of 500 mg.

After eliciting control frequency response curves in both the control and the test preparations, the test preparation was subjected to any one of the following procedures: (i) exposure to NA (3.0 x 10^-3 M) as well as one of the neuron blockers simultaneously for 15 min (5 experiments), (ii) exposure for 15 min to Hukovic solution at 0°C (5 experiments) and (iii) exposure for 15 min to sodium-free Hukovic solution, the osmolarity and the pH of which were maintained with sucrose and KHCO₃ respectively (5 experiments). The neuron blockers under study were then added (except under i) to both the preparations and allowed to act for 15 min. This was followed by 6-8 washouts with Hukovic solution at 35°C. The frequency response curve was then redetermined in both the preparations.

Possible reversal of neuron blockade by various procedures was studied in control preparations. After recording the blocking effects of the neuron blockers in these preparations, they were exposed to either sodium-free solution or to low temp. (0°C) or to NA for 15 min. The preparations were then given 6-8 washouts with Hukovic solution at 35°C followed by redetermination of frequency response curves.

The results are expressed as percentage of maximum responses elicited at 60 Hz during the initial determination of the frequency response curves.

Except when otherwise specified the concentrations of drugs used are given in parentheses. The drugs used were: bretylium tosylate (5.0 x 10^-4 M) (Wellcome Laboratory); debrisoquin sulphate (8.4 x 10^-4 M) (Roche); guanethidine sulphate (3.3 x 10^-5 M) (Ciba); (-)-noradrenaline base (NA; 3.0 x 10^-3 M) (Rhone-Poulenc); xylocholine hydrochloride (1.0 x 10^-8 M) (SK & F).

RESULTS

All the four blockers produced complete block of responses at lower frequencies (3, 5 & 7 Hz). At higher frequencies, debrisoquin and xylocholine produced complete block while guanethidine and bretylium produced only partial block. The neuron block produced by all the four blockers continued for a period of 3-4 hr during which the preparations were as sensitive to NA (5.9 x 10^-6 M) as at the beginning of the experiment. When test preparations were treated with NA, sodium-free Hukovic solution or exposed to low
temp. (0°C), they exhibited increased tone and rhythmic contractions. After equilibrating these preparations with Hukovic solution at 35°C and giving 6 to 7 washes, the initial tone was restored and rhythmic movements disappeared. The responses of vas deferens to nerve stimulation after exposure for 15 min to NA or low temp. or sodium-free solution, were not affected to any appreciable extent.

NA completely prevented the neuron blocking action of all the four blockers at all frequencies (Fig. 1). The reversal of the neuron block due to guanethidine and xylocholine by NA could not be studied after addition of NA as there were persistent spontaneous contractions which continued despite repeated washes with Hukovic solution. Bretylium-induced block was reversed by NA (Fig. 1 d) while debrisoquin-induced block was not.

Exposure of preparations to a low temp. (0°C) prevented the neuron blocking action of guanethidine, bretylium and xylocholine at all the frequencies (3–60 Hz) while that of debrisoquin was partially prevented only at higher frequencies (20 & 60 Hz) (Fig. 2). Low temp. did not reverse the neuron block with any of the four blockers.
FIG. 2. Modifications by low temp. (0°C) of the effects of guanethidine (3.3×10⁻⁵ M; a), debrisoquin (8.4×10⁻⁴ M; b), xylocholine (1.0×10⁻³ M; c) and bretylium (5.0×10⁻⁴ M; d) on contractile responses to hypogastric nerve stimulation at various frequencies (Hz; indicated below each panel) of the paired (control and test) isolated guinea pig hypogastric nerve vas deferens preparations. All responses were recorded after equilibrating the preparations with Hukovic solution at 35°C. Open columns indicate control responses and shaded columns indicate responses elicited after exposure of control preparations to blockers. Diagonal columns indicate the prevention of neuron block in test preparations by low temp. Vertical lines indicate standard errors.

FIG. 3. Modification by sodium-free Hukovic solution of the effect of guanethidine (3.3×10⁻⁵ M) on contractile responses to hypogastric nerve stimulation at various frequencies (Hz; indicated below each panel) of the paired (control and test) isolated guinea pig vas deferens preparations. All responses were recorded after equilibrating the preparations with Hukovic solution at 35°C. Open columns indicate control responses and shaded columns indicate responses elicited after exposure of control preparations to guanethidine. Diagonal columns indicate the prevention of neuron block in test preparations by sodium-deprivation. Vertical lines indicate standard errors.
Prior exposure of preparations to sodium-free Hukovic solution partially prevented only the neuron blocking action of guanethidine (Fig. 3) while that of other three blockers was unaffected. Sodium-free Hukovic solution did not reverse the block induced by any of the four blockers.

**DISCUSSION**

The adrenergic neuron blocking action of guanethidine, xylocholine and bretylium was completely prevented by a low temp. at all frequencies whereas that of debrisoquin was prevented only at higher frequencies. This observation would suggest that the uptake of the former three blockers is completely energy dependant while the uptake of debrisoquin is only partly so. O'Brien & Boullin (17) obtained 97.5% inhibition of uptake of debrisoquin (1 x 10^{-6} M) at 4°C by mammalian platelets which serve as model for the uptake and binding system in noradrenergic nerve endings in the periphery (13). A higher concentration of debrisoquin (8.4 x 10^{-4} M) was used herein. Possibly at high concentrations some debrisoquin passively diffuses into the neuron. This suggestion may be supported by the observation of Reitz et al (18) i.e. a passive diffusion of NA in mouse cerebral cortex slices.

The neuron blocking action of all the blockers was completely prevented by NA, suggesting that these blockers may be competing with NA for uptake into the neuron. If this were true, the neuron blocking action and, therefore, the uptake of all the blockers should have been prevented in a sodium-deficient medium. Sodium deprivation was effective, however, only in the case of guanethidine and failed to prevent the actions of xylocholine, bretylium and debrisoquin. Toda (19) did not observe a preventing action of sodium-deficiency on the adrenergic neuron blocking action of bretylium in rabbit atria and rabbit ascending aorta. O'Brien & Boullin (17) observed only 31.3% inhibition of uptake of debrisoquin by human platelets with ouabain which was shown to inhibit sodium-potassium dependent ATP-ase in human resealed ghost red blood cells (20) and, therefore, reduced extracellular sodium concentration. Since sodium ion is an absolute requirement for the uptake of NA in the adrenergic nerve endings (5, 7, 21) and since the neuron blocking actions of xylocholine, bretylium and debrisoquin were not prevented by sodium deprivation, our results on the ability of NA to prevent the neuron blocking action of these three blockers require explanation. A possibility may be that although these three neuron blockers are taken up by the adrenergic neuron through a mechanism distinct from the one concerned with the uptake of NA, a suggestion already made for bretylium (19, 22), intraneuronally accumulated NA resulting from exposure to high concentrations of NA may not allow effective intraneuronal concentrations of the three neuron blockers to be built up. Debrisoquin, bretylium and xylocholine are known to possess MAO inhibitory action (13, 23, 24). Effective reduction of the intraneuronal concentration of bretylium during exposure to tyramine has been explained as due to a competition between tyramine and bretylium at the enzyme MAO and a number of sites besides the enzyme MAO (23). A recent report has indeed proposed a binding site within the platelets for
debrisoquin, additional to that for 5-HT (17). It is conceivable that high intraneuronal concentrations of NA by precluding occupancy by bretylium, xylocholine and debrisoquin of either the enzyme MAO or other binding sites or both result in a great elevation of the intracellular to extracellular gradient for the free neuron blockers. The net result would be extrusion of free bretylium, xylocholine and debrisoquin from the neuron.

NA reversed the neuron blocking action of bretylium but not that of debrisoquin. Reversal of the neuron blocking action of xylocholine and guanethidine could not be studied for the reasons stated earlier. The transport system of human platelets has greater affinity for debrisoquin than for bretylium (25). After a single injection of debrisoquin to rats, the drug is found in the tissues up to 16 hr in concentrations that inhibit MAO (26). Debrisoquin is about 40 times more potent than bretylium in inhibiting rabbit liver MAO (26). Further, debrisoquin inhibits human platelet MAO noncompetitively (13) and Furchgott et al. (23) have shown with the guinea pig atria that bretylium inhibits the neuronal MAO competitively. An easier displacement of bretylium than of debrisoquin from MAO by NA is, therefore, possible. This would conceivably result in the extrusion of bretylium.

Exposure to sodium-free solution or low temp. did not reverse the neuron blocking action of any of the blockers. This is in accord with the observations of O'Brien and Boullin (17) that the efflux of guanethidine from human platelets is not increased significantly either by ouabain or by iodoacetate and dinitrophenol.

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