MODE OF ACTION OF GUANETHIDINE ON ADRENERGIC NEURONS AND ITS DEPENDENCE ON SODIUM

Takao KUBO* and Yoshimi MISU**

*Department of Pharmacology, Faculty of Pharmaceutical Sciences,
Kyoto University, Sakyo-ku, Kyoto*

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Abstract—Interactions between action of guanethidine (4×10^{-6} to 4×10^{-5}) and Ca, Na or K were investigated in isolated hearts of rabbits. Positive chronotropic responses to electrical sympathetic stimulation and noradrenaline (NA) released by nicotine or high KCl were measured. Inhibitory drug action on responses was irreversible and reduced by raising Ca to 12.5 mM together with drug addition. The action was not reduced after the drug had been washed out and the Ca level was raised to 50 mM, after which it returned to normal. It was accelerated by lowering Na to 86 mM simultaneously, but not delayed by raising Na to 286 mM. After the drug was washed out with normal solution, NA released by 100 and 300 mM KCl was reduced to almost the same extent. When stimulation was done during washing with low Na solution, inhibitory drug action on responses and NA released by nicotine was reduced. It was not reduced, however, when nicotine was applied in normal Na solution after washing with low Na solution. During washing with high Na solution, it was accentuated, despite no modification by washing with isoosmotic sucrose or Li solution. Low or high Na solution alone did not modify the parameters. During washing with low K solution (3.5 mM), it was not modified. The hypothesis is presented that guanethidine increases permeability of nerve ending membrane to Na, thereby leading to adrenergic neuron blockade.

Since the early work of Maxwell et al. (1), the antihypertensive action of guanethidine has been attributed to its adrenergic neuron blockade. Kirpekar et al. (2) demonstrated that in the guanethidine-perfused spleen of cats, high calcium restored the noradrenaline released in response to splenic nerve stimulation. Boullin (3) reported that calcium increased the release of radioactive guanethidine in the heart of rats. On the other hand, it was reported by Wilson (4) and Chang et al. (5) that the antagonism between calcium and guanethidine on contractile responses to sympathetic nerve stimulation was physiological in nature, in the ileum and vas deferens of rats. Boura and Green (6) suggested the possibility that a depressant action of adrenergic neuron blockers in the nerve terminals was analogous to the impairment of conduction in the nerve trunks caused by local anesthetics.

Whether or not an essential interaction exists between the action of guanethidine and calcium, sodium or potassium was investigated herein utilizing the heart of rabbits. The hypothesis has been presented that guanethidine increases the permeability of the

* Present address; Fujisawa Laboratory, Fujisawa Pharmaceutical Co., Ltd., Higashiyodogawa-ku, Osaka, Japan.

** To whom requests for reprints should be addressed.

membrane of nerve endings to sodium ions, thereby leading to adrenergic neuron blockade. Some of these findings were presented at the 38th (7) and 39th (8) Regional Meeting of the Japanese Pharmacological Society, Kinki area.

MATERIALS AND METHODS

About 120 rabbit hearts of both sexes weighing 1.7 to 2.4 kg were used. The bilateral sympathetic trunks (mainly the stellate ganglia) were isolated carefully from the surrounding connective tissues and ligated in the same experimental conditions as described previously by Misu et al. (9). In some experiments, the sympathetic nerve atrial preparations were prepared as described by Misu and Kirpekar (10). The nerves were held by fluid electrodes similar to those described by Garry and Gillespie (11), i.e. a pair of loops of silver wire encased in plastic cell-walls. The preparation was suspended in the muscle chamber containing Krebs bicarbonate solution, bubbled with 95% O₂ and 5% CO₂ maintained at 30°C, having the following composition (expressed in mM): NaCl 118.4; KCl 4.7; CaCl₂ 2.5; MgCl₂ 7 H₂O 1.18; NaHCO₃ 25; KH₂PO₄ 1.2; and glucose 11.1. The final pH was approx. 7.5. Most experiments, however, were done in the Langendorff's preparation, which was prepared as described previously (9). Perfusion was done mainly at a constant pressure of 60 cm H₂O and 30°C. The fluid electrodes were used with a modification for the stimulation of the stellate ganglia in this preparation. Both cells of the electrodes were partitioned with a thin rubber diaphragm. One half of the cell was filled with Krebs solution bubbled with the mixed gas and sealed off with a rubber membrane and a plastic disk with an eyelet of approx. 2 mm diameter in the center. The ligature attached to the stellate ganglia was threaded, by means of a needle, through the eyelet and the rubber diaphragms. The threaded nerves were pulled into the electrodes. The other half cell was then filled with Krebs solution and sealed off with a disk. The thread attached to the stellate ganglia was held between the disk and the casing of the electrodes. The preparation was then suspended from the perfusion apparatus (Natsume KN-206). Spontaneous contraction was recorded on a smoked paper with a semi-isotonic lever. In both preparations of atria and perfused hearts, the bilateral stellate ganglia were stimulated with an electronic stimulator MSE-3 (Nihon Kohden), for 30 sec with supramaximal rectangular pulses of 3.5 msec duration at a frequency of 30 cps. Heart beats/min were counted.

In some perfused hearts, the sympathetic nerve endings were stimulated by perfusion with nicotine solution (4×10^{-5} M) for 2 min or with KCl solution (100 and 300 mM) for 4 min in the same manner as described previously (9). In the latter case, perfusion was performed at a constant volume (10 ml/min) by means of a perfusion pump (Iwashiyu). The output of noradrenaline released into the venous effluent was measured fluorometrically by the method of Anton and Sayre (12) using a spectrofluorophotometer GF-16 (Shimadzu). Recovery was approx. 60%.

A modified Krebs solution containing 215 and 286 mM sodium, 143 mM lithium, 3.5, 23.6, 100 and 300 mM potassium or 12.5 mM calcium was prepared by an addition

or removal of each hydrochlorate without altering the relation of the other electrolytes and compensating changes in tonicity. In order to make a 50 mM calcium Krebs solution, NaHCO_3 was eliminated and pH was adjusted to 7.0 with 1 N KOH. A 86 mM sodium solution was prepared by isoosmotic replacement of sodium chloride (57 mM) by sucrose or glucose.

Drugs used were guanethidine sulphate (Ciba-Geigy), nicotine bitartrate (Tokyo Kasei), cocaine hydrochloride (Takeda) and procaine hydrochloride (Takeda), which are expressed as molar concentrations.

Peak increases of heart rate in responses to electrical stimulation of the sympathetic nerve and the output of noradrenaline released during the perfusion with nicotine or KCl solution before administration of guanethidine are designated as 100% control responses. Student's t-test was used to evaluate data.

RESULTS

Effects of high calcium on guanethidine-induced decrease in positive chronotropic responses to sympathetic nerve stimulation in the isolated atrium of rabbits

Electrical stimulation of sympathetic nerve markedly increased contractile rate and force of the spontaneously beating atria. Percent increases in chronotropic responses were 55.2 ± 8.3 ($n=13$). Guanethidine was added to the organ bath. A concentration of 4×10^{-5} M of the drug produced a slight increase in atrial contractile rate and force. The percent increase in atrial rate was 11.3 ± 1.0 ($n=6$) 30 min after the addition. As demonstrated in Table 1, section B, guanethidine reduced atrial positive chronotropic responses to the 2nd nerve stimulation 30 min after the 1st stimulation to $8.2 \pm 1.3\%$ of control responses. The drug was washed away with normal fresh solution. Responses, which had been reduced by the pretreatment with guanethidine, were not reversible but reduced progressively 30 min after the washing (section C). In section D, the concentration of calcium was raised to totally 12.5 mM together with the addition of guanethidine.

TABLE 1. Effects of high calcium on guanethidine-induced decrease in positive chronotropic responses to electrical stimulation of sympathetic nerve in the isolated atria of rabbit.

Procedures	No. of Exp.	Guanethidine (M)	Calcium (mM)	Positive chrono- tropic responses (mean $\pm$ s.e.) (%)
A. Initial nerve stimulation	13	0	2.5	100
B. In the presence of guanethidine	6	4×10^{-5}	2.5	8.2 ± 1.3
C. After washing out guanethidine	3	4×10^{-5} , then 0	2.5	2.2 ± 1.8
D. In the presence of guanethidine and high calcium	4	4×10^{-5}	12.5	34.6 ± 12.3
E. After washing out high calcium	3	0	50.0, then 2.5	95.3 ± 9.2
F. After incubation first with guanethidine, then with high calcium	3	4×10^{-5} , then 0	50.0 (drug free), then 2.5	0

Stimulation was repeated at intervals of 30 to 60 min

The percent increase in atrial rate was 27.9 ± 8.3 30 min after the addition. Responses to the 2nd nerve stimulation were less inhibited as compared with those after addition of the drug alone. In section E, preparations were incubated with a high calcium solution (totally 50 mM). The high calcium markedly potentiated the atrial contractile force but reduced the rate, sometimes causing a standstill of spontaneous rhythm 10 to 15 min after incubation. The atria, however, were continuously incubated with the high calcium for 30 min, and then were washed with normal calcium solution (2.5 mM). The atrial rate, which had been reduced or abolished by the high calcium, was restored 30 min after the washing and the nerve stimulation produced responses as observed in the control. In section F, the atria were incubated with guanethidine for 30 min, then with the drug-free high calcium solution (50 mM) for 30 min, after which washing with normal calcium solution was carried out. When the 3rd stimulation was carried out in the presence of normal solution 30 min after the washing, responses were nil.

Effect of low or high sodium on guanethidine-induced decrease in positive chronotropic responses to sympathetic nerve stimulation in the isolated heart of rabbits

In untreated perfused hearts, a percent increase in chronotropic responses to the initial nerve stimulation was 42.7 ± 3.4 ($n=3$). As demonstrated in Fig. 1-A, the nerve stimulation produced the same responses as the initial responses at least for 1 hr after perfusion. When hearts were perfused with a solution containing 286 mM sodium, contractile force and positive contractile responses began to decrease markedly 6 to 8 min after the perfusion. Contraction force increased slightly after perfusion with a solution containing 86 mM sodium. Heart rate and positive chronotropic responses, however, did not show marked modifications until at least 20 min after the perfusion with 2 solutions containing modified concentrations of sodium. Thereafter, the responses gradually decreased.

As demonstrated by the solid line in Fig. 1-B, when hearts were perfused with normal sodium solution containing guanethidine (4×10^{-6}), responses to nerve stimulation decreased gradually. Positive chronotropic responses decreased to $28.7 \pm 10.6\%$ of control 30 min after the perfusion ($n=6$). When hearts were washed with fresh solution 30 min after the perfusion, responses were not reversible but reduced progressively. Responses 30 min after washing were $13.0 \pm 5.2\%$ of control ($n=3$). As shown by the bottom line, the inhibitory action of guanethidine was accelerated by perfusion with the low sodium solution (86 mM). The difference was significant ($P < 0.05$) at 10, 15 and 20 min after the perfusion from the action of the drug perfused with normal sodium solution (top line), respectively. The inhibitory action was not delayed or lessened but rather was slightly accelerated by the high sodium solution (middle line).

Perfusion with cocaine or procaine was attempted for comparison with the action of guanethidine. Concentrations of these local anesthetics required to produce similar inhibition of responses to that obtained with guanethidine 4×10^{-6} were found to be 10^{-4} or more, which *per se* markedly depressed contractile rate and force. Responses, depressed by the local anesthetics, were restored almost completely 20 min after washing with fresh solution.

FIG. 1. Effect of low or high sodium on guanethidine-induced decrease in positive chronotropic responses to electrical stimulation of sympathetic nerve in the perfused heart of rabbit.

Stimulation repeated each time is shown in abscissa. Responses to the 1st stimulation are taken as 100% (Figs. 1 and 3-A). After the 1st stimulation, hearts were perfused with solution containing normal (143 mM, —), 286 (----) or 86mM (- - - -) sodium alone (A) and guanethidine (B). Parentheses indicate number of experiments and vertical bars standard errors, these being common to all Figs.

Effect of guanethidine on output of noradrenaline released by high concentrations of KCl in the isolated heart of rabbits

When perfused with 100 or 300 mM KCl solution, hearts did not deteriorate in the hypertonic condition, but occasionally swelling was observed due to coronary constriction. Results obtained in such preparations were omitted. In untreated hearts, the 1st perfusion with 100 and 300 mM KCl solution released 28.2–259.8 ng (an average of 110.4 ng, $n=3$) and 67.8–672.0 ng of noradrenaline (an average of 298.8 ng, $n=3$), respectively.

FIG. 2. Effect of guanethidine on output of noradrenaline released by high concentrations of KCl in the perfused heart of rabbit.

Perfusion with KCl solution was done twice at 30 min intervals. The output of noradrenaline released with the 1st perfusion is taken as 100%, this being common to Fig. 3-B.

In the two columns on the right, the 2nd KCl solution was applied in fresh solution after perfusion with guanethidine solution for 20 min.

As demonstrated in the left half of Fig. 2, the 2nd perfusion with 100 and 300 mM KCl solution released 124.1 ± 7.2 and $109.9 \pm 9.0\%$ of the noradrenaline with the 1st perfusion, respectively. Perfusion with a solution containing equiosmotic amount of glucose to the 300 mM KCl solution did not release a measurable amount of noradrenaline ($n = 2$). The limit of the sensitivity of the present chemical assay of noradrenaline was approx. 10 ng. In the right half, when the 2nd 100 and 300 mM KCl solution was perfused in fresh solution 5 min after washing out the guanethidine solution (4×10^{-6}), the output of noradrenaline was markedly reduced to 37.6 ± 6.1 and $25.7 \pm 2.9\%$ of that released with the 1st perfusion, respectively.

Effect of washing with low or high sodium solution on guanethidine-induced decrease in positive chronotropic responses to sympathetic nerve stimulation in the isolated heart of rabbits

In Fig. 3-A, when the 2nd nerve stimulation was carried out 10 min after washing out the guanethidine solution (4×10^{-6}) with normal sodium solution, responses were $45.0 \pm 4.0\%$ of those obtained with the 1st stimulation (lined column). This value was regarded as control of the drug action, which remained after the drug had been washed out. The action of guanethidine was reduced significantly ($P < 0.05$) by washing with the solution containing 86 mM sodium, being made equiosmotic by addition of sucrose. On the other hand, it was accentuated significantly ($P < 0.01$) by washing with a solution containing 286 mM sodium, but not modified by washing with a solution containing an isoosmotic amount of sucrose (251 mM) or lithium chloride (143 mM).

Effect of washing with low or high sodium solution on guanethidine-induced decrease in output of noradrenaline released by nicotine in the isolated heart of rabbits

In untreated hearts, the 1st perfusion with nicotine solution (4×10^{-5}) released 185.5–2131.5 ng of noradrenaline (an average of 1059.6 ng, $n = 7$). As demonstrated in the ex-

FIG. 3. Effects of washing with low or high sodium solution on guanethidine-induced decrease in positive chronotropic responses to electrical stimulation of sympathetic nerve and in the output of noradrenaline released by nicotine in the perfused heart of rabbit.

Electrical stimulation (A) or perfusion with nicotine solution (B) was carried out twice, usually at 30 min interval. After the 1st procedure, hearts were perfused with guanethidine solution (A, middle and right columns of B) or normal solution (left columns of B) for 20 min, and then washed with washing medium described in abscissa. The 2nd procedure was done during perfusion with washing medium. Horizontal levels of the bracket bar represent the actual % of noradrenaline.

treme left column of Fig. 3-B, the 2nd perfusion with nicotine released $95.3 \pm 14.1\%$ of the noradrenaline obtained with the 1st perfusion. As demonstrated in the left area of Fig. 3-B, when the 2nd nicotine solution was perfused 5 min after washing out the drug-free solution with 286 mM and 86 mM sodium solution, being made equiosmotic by addition of glucose (120 mM), the output of noradrenaline was 84.9 ± 13.1 and $92.6 \pm 16.3\%$ of that released with the 1st procedure, respectively.

When the 2nd nicotine solution was perfused into the solution containing guanethidine 2×10^{-5} 30 min after the perfusion, the output of noradrenaline was markedly reduced

(3.6%, $n=2$). When the 2nd nicotine solution was perfused to normal sodium solution 5 min after washing out the guanethidine solution, however, the output of noradrenaline was reduced to $35.5 \pm 1.7\%$ of that released with the 1st procedure (lined column). This value was regarded as control of the drug action, which remained after the drug had been washed out. The action of guanethidine was significantly ($P < 0.01$) accentuated by washing with 286 mM sodium solution ($8.8 \pm 1.3\%$), as well as being moderately accentuated with 215 mM sodium solution (23.8%). On the other hand, it was significantly reduced ($P < 0.01$) by washing with 86 mM sodium solution ($66.0 \pm 4.0\%$).

Hearts were washed with 86 mM sodium solution for 5 min, and then perfused with normal sodium solution as demonstrated in the extreme right column of Fig. 3-B. When the 2nd nicotine solution was perfused 5 min after, the output of noradrenaline was $29.4 \pm 2.6\%$ of that released with the 1st nicotine perfusion. This value did not differ from control regarding the action of guanethidine.

Effect of washing with low or high potassium solution on guanethidine-induced decrease in output of noradrenaline released by nicotine in the isolated heart of rabbits

When the 2nd nicotine solution was perfused into a low potassium solution (3.5 mM) 5 min after washing out the drug-free solution (potassium 4.7 mM), the output of noradrenaline was $61.1 \pm 5.0\%$ ($n=4$) of that released with the 1st nicotine perfusion, this difference being significant ($P < 0.05$) from that in untreated hearts ($95.3 \pm 14.1\%$, the extreme left column in Fig. 3-B). The low potassium could cause hyperpolarization of the nerve membrane and antagonize the sustained depolarization induced by nicotine (13). When the 2nd nicotine solution was added to the low potassium solution 5 min after washing out the guanethidine solution (2×10^{-6}), the output of noradrenaline was reduced to $25.1 \pm 2.7\%$ ($n=4$) of that released with the 1st nicotine perfusion, the difference being also significant ($P < 0.05$) from the control regarding the action of the drug ($35.5 \pm 1.7\%$, lined column in Fig. 3-B).

On the other hand, when the 2nd nicotine solution was perfused while the hearts were being perfused with a high potassium solution (23.4 mM) 5 min after washing out the drug solution, the output of noradrenaline was $36.6 \pm 2.5\%$ ($n=3$) of that released with the 1st nicotine perfusion, the difference thus being insignificant.

DISCUSSION

The adrenergic neuron blocking action of guanethidine was reduced when the calcium level was raised simultaneously with the drug addition. The result is consistent with the findings reported by other investigators (4, 5, 14). When nerve stimulation is done in the presence of high calcium, however, a simple interaction between calcium and guanethidine should be taken into account, as high calcium increases noradrenaline released from the sympathetic nerve (15, 16) and the drug reduces noradrenaline (2, 7, 17). The present result may be attributed to a physiological antagonism between calcium and guanethidine on the output of the transmitter. In order to clarify the essential interaction other

than the antagonism, nerve stimulation should be done in the presence of normal calcium after washing out high calcium (2, 5). Atrial responses to sympathetic nerve stimulation, which had been reduced by the pretreatment with guanethidine (4×10^{-5} M), were not reversed when the drug was washed out and the concentration of calcium was raised to 50 mM for 30 min, after which the calcium concentration returned to normal. The irreversibility of the action of the drug was not due to a marked inhibition of the high calcium itself on heart rate, which appears to be a membrane stabilizing action (18). The result is not consistent with the findings reported by Kirpekar et al. (2) that the guanethidine (1.2×10^{-6})-induced abolition of noradrenaline released by stimulation of the splenic nerve of cat was restored to approx. 50% of control when the drug was washed out and calcium concentration was raised to 50 mM for 20 to 30 min, after which the high calcium concentration returned to normal. This inconsistency may be derived from differences in doses of the drug, parameters of experiments, organs or species. Various findings have been reported by different investigators in reference to interactions between the action of guanethidine and calcium (2-5, 14). No essential interaction between the action of guanethidine 4×10^{-5} and 50 mM calcium was found in the parameter of atrial positive chronotropic responses to nerve stimulation in rabbits.

Boura and Green (6) attributed the mode of action of adrenergic neuron blockers to a conduction blockade in the nerve terminals. The hypothesis was supported by Haeusler et al. (19, 20). The present results, however, show that the situation is indeed more complex. Hodgkin and Katz (21) demonstrated that in squid giant axon, the reversed potential difference of the active membrane depended on the external sodium concentration, that the conduction velocity underwent a decrease in low sodium solution and that the height of the action potentials was increased by a solution containing additional sodium chloride. Thus, if the conduction blockade induced by guanethidine is a major factor for its adrenergic neuron blockade, then the blocking action of the drug would be accentuated by lowering sodium and reduced by raising the ion. In fact, the adrenergic blockade induced by guanethidine was accelerated by perfusion with the solution containing low sodium (86 mM) together with the drug addition, within the time when the low sodium itself produced no decrease in responses to nerve stimulation. These results partially support the above-mentioned hypothesis (6, 19, 20). It should be noted, however, that nerve stimulation was carried out while perfusion with the solution containing guanethidine was being given. On the other hand, the action of the drug was not delayed or reduced by simultaneous perfusion with the high sodium (286 mM), the same of which can not be attributed to hypertonicity of the solution. It was confirmed that in the present preparations, a complete adrenergic blockade was induced by tetrodotoxin (6.2×10^{-8}) 15 min after the application, and was significantly ($P < 0.05$) delayed by the simultaneous perfusion with the high sodium (8). There is the case that acute partial sympathetic blockade by reserpine was reduced by perfusion with high sodium (9). These results provided the speculation that guanethidine itself may have an action against adrenergic blockade due to its local anesthetic action, and the fact of no reduction of the guanethidine action

induced by the high sodium could be attributed to being in the balance between the dual actions of the drug. The dissociation of the primary action of guanethidine from its local anesthetic action is reported to have been observed in the sympathetically innervated organs and the sensory C fibers (22, 23).

On the other hand, Haeusler et al. (20) have demonstrated in the heart of cats, that when guanethidine blocked the firing normally induced by a lower concentration of acetylcholine, antidromic discharges could still be obtained by a higher concentration of acetylcholine, and concluded that the adrenergic neuron blocking action of guanethidine may well be explained by the combination of its local anesthetic action and its accumulation in nerve terminals. They applied acetylcholine while perfusing with guanethidine. In the present experiments, high concentrations of KCl were applied to induce depolarization of the nerve membrane after guanethidine in extra- or intracellular space to be easily washed away had been washed out. The action of guanethidine was not reversible but reduced progressively after washing out, which differed from that of high doses of cocaine and procaine. As reported previously (9), reserpine (1.6×10^{-6}) markedly reduced noradrenaline released by 100 mM KCl, but not reduction was seen with 300 mM KCl in the same experimental design. Different from the action of reserpine, however, guanethidine did not produce a 'ceiling effect' of the decrease in noradrenaline released by the higher concentration of 300 mM KCl. It is feasible that the action of guanethidine which remained after the drug had been washed out is not due to an antagonistic action against depolarization of the nerve membrane induced by KCl, i.e. it is not due to a membrane stabilizing action. This experimental design can be applied to further investigations in order to clarify the action of guanethidine itself against its local anesthetic action.

Guanethidine-induced decreases in positive chronotropic responses to electrical stimuli and noradrenaline released by nicotine were reversed by subsequent perfusion with the solution containing low sodium (86 mM). There is much evidence supporting the fact that sodium is required for the uptake and storage of noradrenaline by the sympathetic nerve endings (24-27). One possible explanation for the results presented herein is that a decrease in the re-uptake of noradrenaline induced by the perfusion with the low sodium resulted in an increase in the recovery of the transmitter. This is hardly feasible, however, since the output of noradrenaline was not potentiated by the perfusion with the low sodium solution alone.

There is the possibility that restoration from the action of guanethidine can be attributed to an easier removal of the drug from a site of action by an unknown mechanism in a specific condition of the low sodium, for example, as sodium deficient media produce an increase in efflux of radioactive noradrenaline from prelabelled heart slices (25-27). The result herein (the extreme right column in Fig. 3-B), however, shows that such is not the case. The inhibitory action of guanethidine on the output of noradrenaline reappeared when nicotine was added to normal sodium solution after hearts had been perfused with the drug solution, and then with the drug-free low sodium solution. A decreased amount of guanethidine would hardly result in the reappearance of its action.

Guanethidine is taken up into the sympathetic nerve endings by the same uptake process for noradrenaline (28-30). Gulati and Jaykar (31) reported the possibility that in the isolated rabbit's ileum, prevention from adrenergic neuron blockade could be due to the decrease in the uptake of guanethidine itself caused by previous or simultaneous incubation with a sodium free medium. This does not, however, explain the restoration from the action of guanethidine in the present experiment, induced by the subsequent perfusion with the low sodium solution.

Guanethidine-induced decreases in responses to electrical stimuli and noradrenaline released by nicotine were accentuated by the subsequent perfusion with the high sodium solutions. The potentiating effect of the high sodium was not due to hypertonicity of the solution but rather to the action of sodium itself. Lithium was not effective in replacing the action of sodium.

From an above-mentioned relationship between the concentrations of sodium and the action of guanethidine, a probable explanation is that guanethidine increases the permeability of the membrane of the endings to sodium ions, thereby leading to adrenergic neuron blockade. Such potency of guanethidine was demonstrated in the membrane of the isolated skin of frogs (32) and of atria isolated from rabbits pretreated with reserpine (33). Misu (33) demonstrated a biphasic action of guanethidine 4×10^{-4} on the transmembrane potential of left driven atria. The maximum rate of rise of action potential significantly increased without hyperpolarization up to 30 min after its application, and then the resting potential gradually decreased. The former action of guanethidine was blocked by the pretreatment with 1.6×10^{-7} and the latter action was inhibited by the addition of 3.1 and 6.2×10^{-6} of tetrodotoxin.

Keen and Bogdanski (27) have suggested an inhibitory and antagonistic action of sodium to calcium on a postulated receptor site in the release of noradrenaline. Since there is presumably no essential interaction between the action of guanethidine and calcium, it would be feasible that guanethidine increases the permeability of the membrane of the adrenergic nerve terminals to sodium ions and increases the concentration or availability of sodium to compete with calcium at some site in the process by which the transmitter is released. Perfusion with a low potassium solution did not modify the action of guanethidine.

It is noteworthy that the drug is particularly of therapeutic value in the treatment of hypertensive patients, where the sodium level in extracellular fluid is highly elevated.

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