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REVERSAL OF GUANETHIDINE BLOCKADE OF SYMPATHETIC NERVE TERMINALS BY TETRAETHYLAMMONIUM AND 4-AMINOPYRIDINE

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1 The effect of tetraethylammonium (TEA) and 4-aminopyridine (4-AP) on the inhibitory effect of guanethidine on noradrenaline (NA) release was investigated in the perfused spleen of the cat.

2 Guanethidine blocked the release of NA evoked by nerve stimulation. TEA and 4-AP readily reversed this inhibitory effect, and the NA output was nearly doubled after repeated stimulation of the nerves. On subsequent perfusion with Krebs solution without TEA or 4-AP, the inhibitory effect of guanethidine reappeared.

3 The reversal of guanethidine blockade of sympathetic nerves by TEA and 4-AP is discussed.

Introduction

The mechanism by which guanethidine inhibits the release of noradrenaline (NA) from sympathetic nerves evoked by electrical stimulation is not yet fully understood. It has been proposed that guanethidine and also bretylium accumulate intraneuronally and act as a local anaesthetic on the membrane of the sympathetic nerve terminals to inhibit NA release (Haeusler, Thoenen, Haefely & Huerlimann, 1968; Haeusler, Haefely & Huerlimann, 1969). An alternative suggestion was that guanethidine depresses NA release by limiting the access of calcium to those sites in the postganglionic sympathetic nerve ending with which calcium interacts to cause the release of NA (Kirpekar, Wakade, Dixon & Prat, 1969). The present investigation was undertaken to test this theory and to determine whether tetraethylammonium (TEA) and 4-aminopyridine (4-AP), which enhance NA output, presumably by increasing calcium entry into the sympathetic nerves (Thoenen, Haefely & Staehelin, 1967; Kirpekar, Prat, Puig & Wakade, 1972; Kirpekar, Kirpekar & Prat, 1976a; Kirpekar, Wakade & Prat, 1976b), reverse the neuronal blockade produced by guanethidine. A preliminary account of some of these findings has been published (Kirpekar, Kirpekar & Prat, 1977).

Methods

Cats (about 2 kg) were anaesthetized with ether followed by chloralose (60 mg/kg, i.v.). The arrangements for the perfusion of the spleen *in situ* were similar to those described by Kirpekar & Misu (1967). The spleen was perfused with Krebs-bicarbonate (KB) solution at 35°C by means of a pump (Sigmamotor, Model A14E) at a constant rate of about 7 ml/minute. A mixture of 95% O_2 and 5% CO_2 was bubbled through the KB and the final pH was 7.4 to 7.5. The splenic nerves were stimulated with rectangular pulses (25 V, 1 ms) at a frequency of 10 Hz for 20 seconds. Samples were collected from the splenic vein, each for 2 min, beginning with the start of stimulation. The perfusion pressure was used as a measure of peripheral resistance and was recorded. The NA content of the samples was determined fluorometrically by the method of Anton & Sayre (1962).

The spleen was initially perfused with normal KB for 30 min, followed by a KB containing guanethidine (0.3 μg/ml) for 15 minutes. After establishing the inhibitory effect of guanethidine on NA release, the perfusion was continued with KB containing TEA or 4-AP (1 mM) for about 60 min, and then with normal KB for an additional 30 to 60 minutes. During the different perfusion periods the nerves were stimulated at intervals of about 15 minutes.

Results

The amount of NA released from the perfused cat spleen remained fairly constant during different periods of stimulation. (Kirpekar, *et al.*, 1976b). At a stimulation frequency of 10 Hz the quantity of NA released during the 8th stimulation period amounted

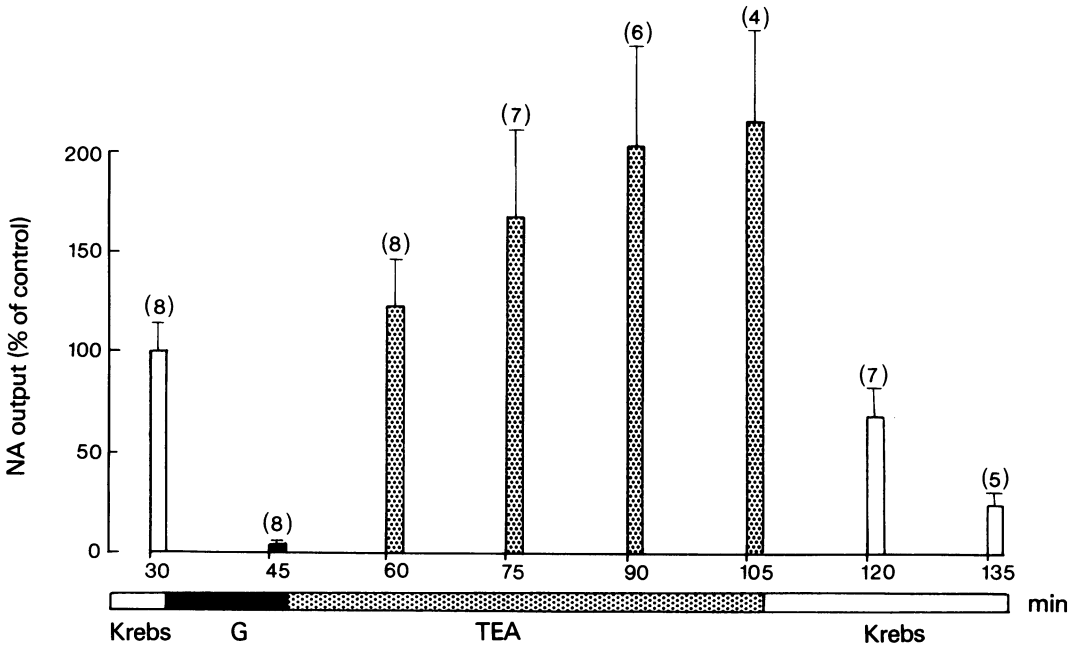

Figure 1 Effect of tetraethylammonium (TEA) on the inhibitory effect of guanethidine (G) on noradrenaline (NA) release from the *in situ* perfused spleen of the cat. Splenic nerves were stimulated at 10 Hz for 20 s; NA release is expressed as percentage (mean) of the initial release (= 100%); 1st open column: initial release (115 ± 18 ng per 2 min); closed columns: perfusion with guanethidine (0.3 μg/ml; stippled columns: perfusion with TEA (1 mM). Vertical lines show s.e. mean. Numbers in parentheses: number of observations.

to about 70 to 80% of that released during the initial stimulation period. It has been reported previously that the inhibitory effect of guanethidine on the release of NA induced by nerve stimulation persists for several hours, even after removal of guanethidine from the perfusion fluid (Thoenen, Huerlimann & Haefely, 1966). A spleen which had first been perfused with guanethidine and subsequently for nearly 2 h with guanethidine-free KB, released in response to nerve stimulation at a frequency of 30 Hz only 10 ± 4% (n = 4) of the amount of NA released during the initial control period (Kirpekar *et al.*, 1969). In the present experiments a stimulation frequency of only 10 Hz was used and therefore 2 experiments were carried out to study the duration of the inhibitory effect of guanethidine on NA release evoked by nerve stimulation at 10 Hz. The spleen was first perfused with guanethidine and then for 90 min with a guanethidine-free solution. At the end of this perfusion the NA release following nerve stimulation was only 15% of the control value.

Figure 1 shows the reversal by TEA of the inhibitory effect of guanethidine on NA release. Guanethidine suppressed NA release induced by nerve stimulation by about 90%. After 15 min perfusion with KB containing TEA (1 mM) the NA output was restored to the initial control output. As perfusion with TEA continued, the NA output increased gradually at each subsequent stimulation. After 1 h it was about double the control value. Perfusion pressure responses to nerve stimulation followed roughly the same pattern as NA release. Thus, after perfusion with guanethidine the perfusion pressure responses were greatly suppressed, whereas perfusion with KB containing TEA not only restored the pressure responses fully, but even potentiated them. On subsequent perfusion with KB without TEA, the mean amounts of NA released in response to the first and second stimulation decreased gradually to 70% and to 25% of the initial control values. In one experiment, stimulations were repeated again twice and the NA output was only 16% of the control value. On reperfusion with KB containing TEA the NA release was again enhanced and values nearly twice the initial control values were reached. This experiment shows that the releasable store of NA did not become exhausted on repeated nerve stimulation. The corresponding perfusion pressure responses in KB were reduced, but not in pro-

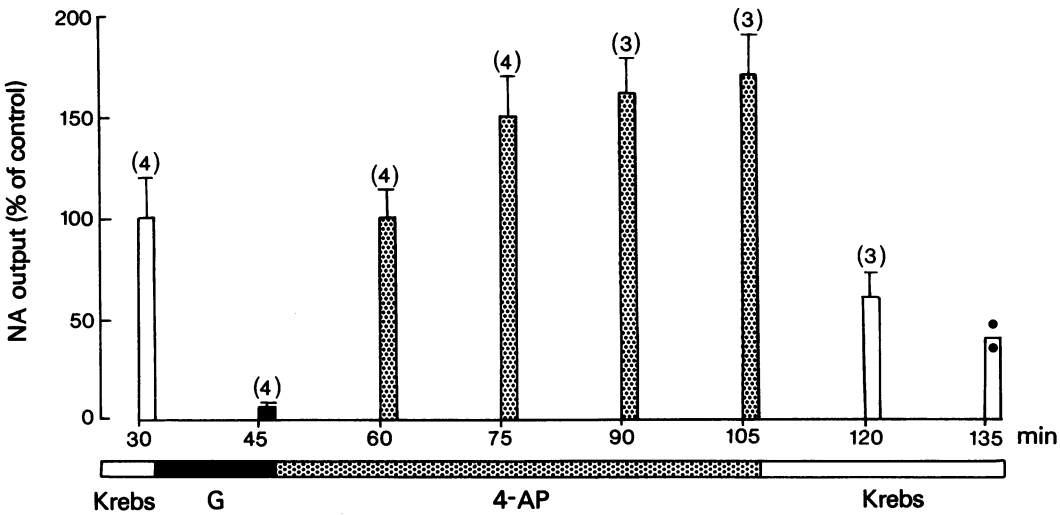

Figure 2 Effect of 4-aminopyridine (4-AP) on the inhibitory effect of guanethidine (G) on noradrenaline (NA) release from the *in situ* perfused spleen of the cat. Splenic nerves were stimulated at 10 Hz for 20 s; NA release is expressed as percentage (mean) of the initial release (= 100%); 1st open column: initial release (152 ± 32 ng/2 min); solid column: perfusion with guanethidine (0.3 μg/ml); stippled column: perfusion with 4-AP (1 mM). Vertical lines show s.e. mean. Numbers in parentheses: number of experiments.

portion to the reduction of NA output. These experiments show also that TEA only temporarily restores the function of adrenergic nerve terminals after guanethidine blockade.

Figure 2 shows the effect of 4-AP in reversing the inhibition of NA release by guanethidine. The reduced NA release after guanethidine was restored to the initial value during perfusion with KB containing 4-AP for 15 minutes. As with TEA, NA release increased gradually on each subsequent stimulation, and was almost double the initial value during the 3rd and 4th stimulation period. The responses of the perfusion pressure to stimulation were suppressed by guanethidine and greatly enhanced during the perfusion with 4-AP. On subsequent reperfusion with KB without 4-AP, the mean amounts of NA released in response to the first and second stimulation were 60 and 40% of the initial values. In 2 experiments, stimulations were repeated again twice, and the outputs of both periods were about 30% of the control value; on reperfusion with 4-AP the NA release was again enhanced to values about twice the initial control value. These experiments show that, like TEA, 4-AP also temporarily reverses the guanethidine blockade of NA release. The enhancement of NA release from control spleens by 4-AP is not as readily reversible as that occurring with TEA (unpublished observations). This fact may partly account for the greater restorative effect of 4-AP as compared to TEA during the final perfusion.

Discussion

Experiments described in this paper show that TEA and 4-AP dramatically reversed the inhibitory effect of guanethidine on NA release from the spleen by nerve stimulation. This reversal of the blockade of nerve transmission by TEA and 4-AP could be brought about by displacement of guanethidine from the sympathetic nerves by TEA and 4-AP. However, TEA or 4-AP only temporarily restored noradrenaline release after blockade with guanethidine. Another possibility is that TEA or 4-AP simply restored the conduction block at the terminal portion of the sympathetic nerve ending. Since we are not aware that TEA or 4-AP can restore conduction in blocked nerves we cannot comment on this point. A third possibility is that TEA and 4-AP may enhance calcium entry into the sympathetic nerves to reverse guanethidine blockade of NA release. TEA and 4-AP greatly enhance NA release by splenic nerve stimulation (Thoenen *et al.*, 1967; Kirpekar *et al.*, 1972; 1976a; 1976b), and this appears to be mainly due to the ability of TEA and 4-AP to inactivate potassium current and prolong the duration of the action potential, thereby allowing a greater influx of calcium ions into the neurone. Katz & Miledi (1969) have shown that TEA increases transmitter release, presumably by enhancing the calcium entry into the nerve terminal in a regenerative way. It is therefore possible that TEA and 4-AP also enhance the calcium entry into

the postganglionic sympathetic nerve terminals during the course of an action potential. If these ions enhance calcium entry during nerve stimulation, even in the presence of guanethidine, then it is conceivable that the greater influx of calcium was probably responsible for reversing the inhibitory effect of guanethidine on NA release. Gillespie & Tilmisany (1976) have recently shown that TEA reversed the inhibitory effect of guanethidine on the responses of the rat anococcygeus muscle to motor nerve stimulation. They have suggested that the antagonism between guanethidine and TEA probably involves mobilization of calcium within the nerve membrane.

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