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EFFECTS OF AMPHETAMINE ON THE RESPONSES OF THE GUINEA-PIG ISOLATED VAS DEFERENS PREPARATION TO HYPOGASTRIC NERVE STIMULATION

BY

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In concentrations from 1 to 100 μg/ml., amphetamine potentiated responses of the isolated vas deferens preparation of the guinea-pig stimulated via the hypogastric nerve at rates of from 5 to 40 shocks/sec. Potentiation was more pronounced with low than with high stimulus frequencies. A concentration of 500 μg/ml. of amphetamine had a blocking action which was more pronounced at high than at low stimulus frequencies. This effect contrasted with the ganglionic blocking action of hexamethonium which had a greater effect at low than at high stimulus frequencies.

The interaction between guanethidine and dexamphetamine has been studied by Day & Rand (1963) on several tissues including the vas deferens preparation. The present work describes the effect of amphetamine on responses to various rates of stimulation of the guinea-pig isolated hypogastric nerve-vas deferens preparation.

METHODS

The guinea-pig vas deferens with hypogastric nerve was set up according to the method of Huković (1961) except that the distal end of the vas deferens was fixed to the glass oxygenation tube so that the platinum ring electrodes (Burn & Rand, 1960) carrying the nerve could be placed in close proximity to the muscle without interfering with contractions. The length of nerve between the muscle and the electrodes was about 0.5 cm; these are termed "close electrodes." In some experiments the nerve was also threaded through a second pair of electrodes at 2 cm from the muscle; these are termed "distant electrodes." The preparation was mounted in a 10 ml. organ-bath containing Krebs solution (composition in g/l. of distilled water: NaCl 6.92, KCl 0.354, $NaHCO_3$ 2.1, KH_2PO_4 0.162, $MgSO_4$ 0.293, $CaCl_2$ 0.28 and dextrose 2.0) maintained at 31° C and bubbled with a mixture of 5% carbon dioxide and 95% oxygen. The bath was washed by overflow and recordings were made by means of a frontal writing lever on smoked paper.

The nerve was stimulated by rectangular pulses of 2 msec duration and of supramaximal voltage. Trains of 25 or 100 shocks were delivered at rates of from 5 to 40 shocks/sec every 2 min.

Concentrations of amphetamine sulphate, ephedrine sulphate, hexamethonium bromide and tyramine hydrochloride refer to the salts. Concentrations of noradrenaline bitartrate are expressed in terms of the base.

RESULTS

Responses to nerve stimulation

When the nerve was stimulated with 100 shocks at a frequency of 5 shocks/sec, there was a latent period of a few seconds before the preparation responded with a discontinuous stepwise contracture. Higher stimulus frequencies gave more rapidly developing and greater responses. When 25 shocks were given, the response to 40 shocks/sec was slightly less than that to 20 shocks/sec (Fig. 1, *a*). Stimulation

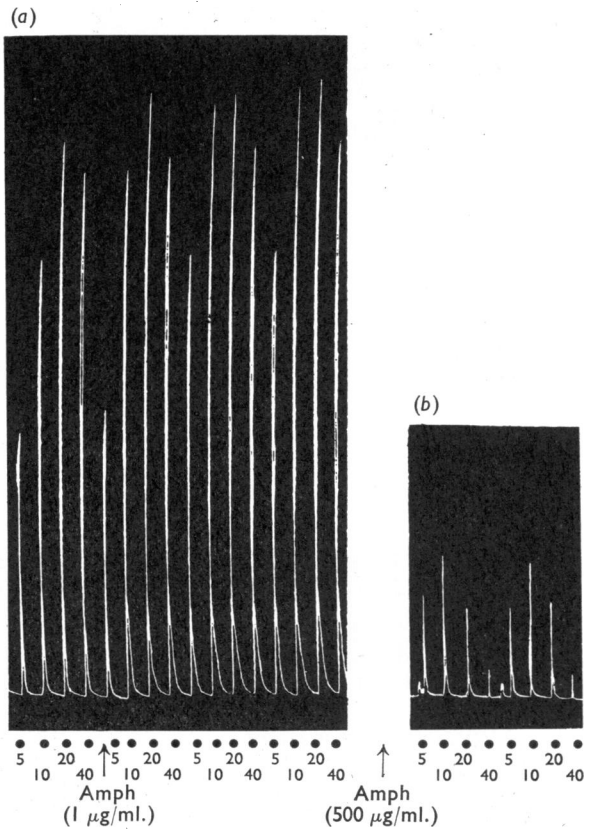

Fig. 1. Responses of a guinea-pig isolated vas deferens preparation to "close" stimulation of the hypogastric nerve with 25 supramaximal shocks at 5, 10, 20 and 40 shocks/sec (numbers below records) at 2 min intervals. In (*a*), 1 μg/ml. of amphetamine (Amph) was given at the arrow. Between (*a*) and (*b*), 500 μg/ml. of amphetamine were added to the bath, and (*b*) shows responses 33 min later.

with 100 shocks at 5 shocks/sec applied through "distant" electrodes was ineffective in one experiment and gave small or diminishing responses in three others. Responses at all rates were greater when stimuli were applied through the "close" compared with through the "distant" electrodes. After stimulation through the "close" electrodes, the effect of "distant" stimulation was diminished or was sometimes absent. This effect was more marked with low than with high stimulus frequencies (Fig. 3, *a*).

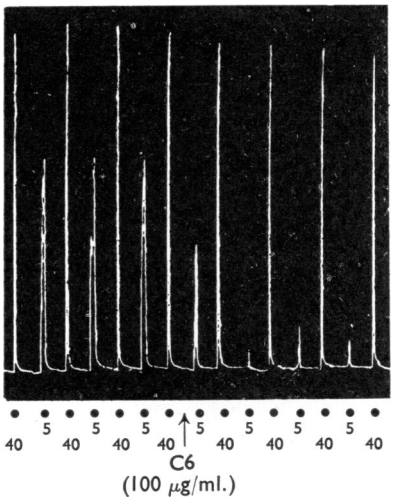

Fig. 2. Responses of a guinea-pig isolated vas deferens preparation to " distant " stimulation of the hypogastric nerve with 100 supramaximal shocks at 5 and 40 shocks/sec (numbers below record) at 2 min intervals. At the arrow, hexamethonium (C6, 100 μg/ml.) had little effect on responses to stimulation at 40 shocks/sec but almost completely blocked responses to 5 shocks/sec.

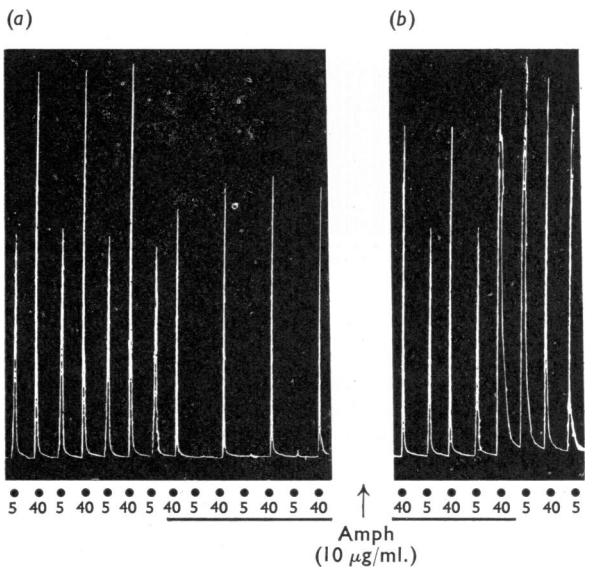

Fig. 3. Responses of a guinea-pig isolated vas deferens preparation to stimulation of the hypogastric nerve with 100 supramaximal shocks at 5 and 40 shocks/sec (numbers below records) at 2 min intervals. The horizontal bars indicate stimulation through " distant " electrodes. The other responses are with " close " electrodes. Between (*a*) and (*b*), amphetamine (Amph, 10 μg/ml.) was added to the bath fluid, and (*b*) shows responses 15 min later.

Effects of hexamethonium

Hexamethonium (100 μg/ml.) blocked or greatly reduced the effect of stimulation through "distant" electrodes. If the nerve was then pulled through the electrodes so that the electrodes were close to the vas, or if stimulation was applied through a second pair of electrodes close to the muscle, the response to stimulation was undiminished. These observations accord with those of Ohlin & Strömblad (1963) and of Bentley & Sabine (1963). The blocking effect of hexamethonium was more pronounced on the responses to low than on those to high stimulus frequencies (Fig. 2), as was found by Sjöstrand (1962).

Effects of amphetamine

Concentrations of amphetamine from 1 to 100 μg/ml. potentiated responses to nerve stimulation, the effect being greater with low than with high frequencies. Fig. 1, *a* shows the effect of 1 μg/ml. when 25 shocks were delivered through "close" electrodes at various rates, the responses to 5, 10, 20 and 40 shocks/sec being increased by 68, 41, 11 and 5% respectively after 10 min exposure to amphetamine. Fig. 3 shows the effect of 10 μg/ml. when 100 shocks were applied through "close" and "distant" electrodes at 5 and 40 shocks/sec. The potentiating effect of amphetamine on the low rate using "distant" electrodes was particularly marked.

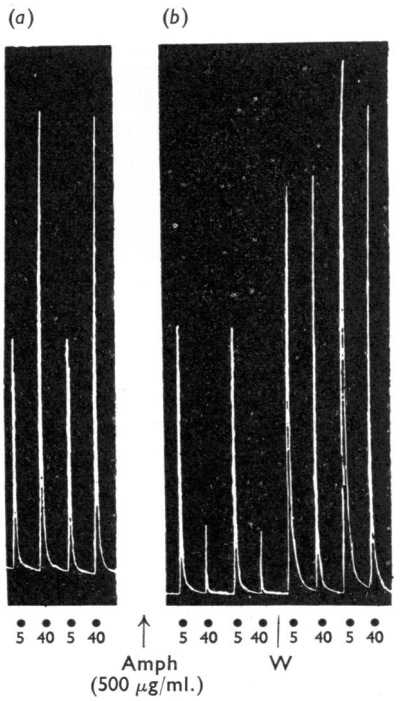

Fig. 4. Responses of a guinea-pig isolated vas deferens preparation to "close" stimulation of the hypogastric nerve with 25 supramaximal shocks at 5 and 40 shocks/sec (numbers below records) at 2 min intervals. Between (*a*) and (*b*), amphetamine (Amph, 500 μg/ml.) was added to the bath, and (*b*) shows the responses 31 min later. The preparation was washed twice at W.

High concentrations of amphetamine (500 μg/ml.) antagonized the responses to high stimulus frequencies to a greater extent than those to low rates. In Fig. 1, *b* the responses to trains of 25 shocks at 5, 10, 20 and 40 shocks/sec were reduced to 38, 32.5, 16 and 5% respectively of the initial heights shown in Fig. 1, *a*. Fig. 4 shows that whereas initially trains of 25 shocks at 40 shocks/sec gave responses twice the height of those produced by 5 shocks/sec, the responses to 40 shocks/sec after amphetamine (500 μg/ml.) were only 25% of those to 5 shocks/sec. A similar relationship held for 100 shocks at high and low stimulus frequencies. The blocking action of amphetamine was rapidly reversed on washing out (Fig. 4), the responses then showing the persistent potentiation regularly seen after washing following lower concentrations of the drug.

Tyramine and ephedrine (1 to 100 μg/ml.) also potentiated the responses to nerve stimulation, but had no blocking action in concentrations of 500 and 400 μg/ml. respectively.

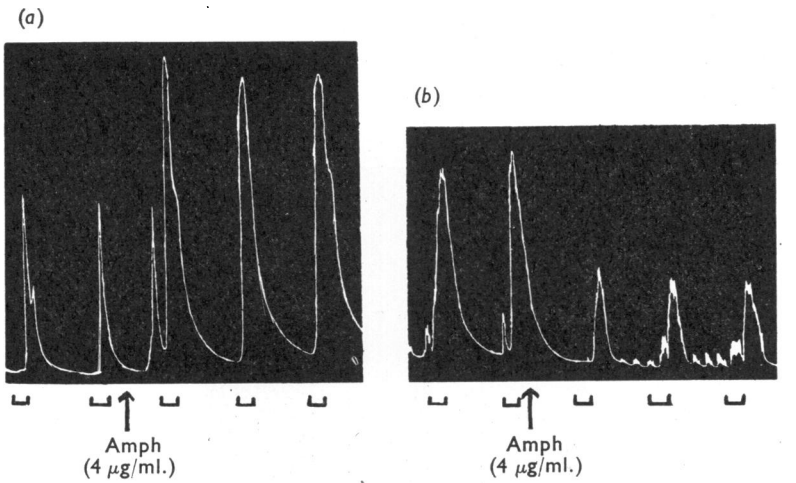

Fig. 5. Responses of a guinea-pig isolated vas deferens preparation to noradrenaline (2 μg/ml.) added to the bath fluid for 45 sec periods (during the marks) at 4 min intervals. In (*a*), at the arrow, amphetamine (Amph, 4 μg/ml.) potentiated responses. In (*b*), at the arrow, amphetamine (500 μg/ml.) reduced responses, contractures occurring only on washing out the noradrenaline doses.

In view of the foregoing results, the effects of amphetamine on the responses of the vas deferens to noradrenaline were examined. After adding noradrenaline to the bath fluid, there was a latent period of 20 to 30 sec before contraction commenced. Fig. 5, *a* shows the potentiating effect of amphetamine (4 μg/ml.) and Fig. 5, *b* shows the blocking action of 500 μg/ml. of amphetamine on responses to 2 μg/ml. of noradrenaline. During the presence of this high concentration of amphetamine, the preparation gave a contracture only after washing the prepara-

tion. This " wash-out " response is usually seen with subthreshold concentrations of stimulant drugs (Ohlin & Strömblad, 1963).

DISCUSSION

Day & Rand (1963) reported that low concentrations of dexamphetamine had a slight potentiating action on the responses of the guinea-pig isolated vas deferens stimulated via the hypogastric nerve. They used a stimulus frequency of 20 shocks/sec applied for 5 sec. Burn & Weetman (1963) found, as I have, that this stimulus frequency gives maximal responses. With lower stimulus frequencies, the poteniating effect of amphetamine was pronounced (Figs. 1 and 3).

The blocking action of high concentrations of dexamphetamine was attributed by Day & Rand (1963) to a guanethidine-like effect. My results indicate that the blocking activity of amphetamine is no more than one-fifth of that found by these authors for dexamphetamine. On the cat nictitating membrane response to post-ganglionic nerve stimulation, Day & Rand (1963) found that large doses of dexamphetamine produced a roughly parallel shift to the right in the stimulus frequency/response curve, whereas my work shows that the blocking effect of amphetamine on the responses of the vas deferens is absolutely as well as relatively greater for higher than for lower stimulus frequencies (Figs. 1 and 4). On the other hand, the results described here (Fig. 2) and those of Sjöstrand (1962) show that hexamethonium has a more pronounced effect on low than on high stimulus frequencies, although there is now good evidence that this drug acts at a conventional ganglionic synapse (Ohlin & Strömblad, 1963). Burn, Dromey & Large (1963) have suggested that, at low stimulus frequencies, acetylcholine as well as noradrenaline is released, whereas at high rates only noradrenaline is released to act on the muscle. If high concentrations of amphetamine prevent the release of noradrenaline this could account for my observations on nerve stimulation. However, high concentrations of amphetamine also antagonize the action of noradrenaline added to the bath fluid (Fig. 5, *b*) and my findings neither directly support nor refute the postulates of Burn *et al.* (1963).

The inhibitory action of stimulation of the hypogastric nerve close to the vas deferens upon subsequent distant stimulation is puzzling. Physical trauma can hardly be implicated, for amphetamine reinstated the response to distant stimulation (Fig. 3).

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