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Research Papers

Influence of temperature on the responses of the guinea-pig hypogastric nerve-vas deferens preparation

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Over a temperature range of 32 to 20°, responses of the vas deferens to hypogastric stimulation at 50 shocks/sec became gradually smaller, while those to 10 shocks/sec increased gradually. A parallel study, under the same temperature conditions, of drugs known to interfere with peripheral sympathetic transmission revealed a marked reduction of the inhibitory properties of the adrenergic neurone blocking agents bretylium and xylocholine at the lower temperatures. The mechanism by which cooling modified the responses of the preparation to nerve stimulation as well as to the activity of the drugs on the vas is discussed and tentative hypotheses are advanced.

IN recent years the Huković (1961) preparation of the guinea-pig isolated vas deferens has been widely used for the study of peripheral sympathetic transmission and of its modifications by drugs. Apart from the observations of Kuriyama (1964), which concern only the junction potentials produced at different temperatures by hypogastric nerve or muscle field stimulation, we are unaware of any report on the influence of temperature either on the responses of the vas to different stimulation frequencies, or, with the exception of the work of Leach (1956), on the pharmacological activity of known drugs on the vas deferens.

The present paper describes experiments of this kind.

Methods and materials

HYPOGASTRIC NERVE-VAS DEFERENS PREPARATION

Guinea-pigs weighing 350–500 g were used. The preparation, as described by Huković (1961), was set up in a 100 ml organ bath containing Krebs solution gassed with carbon dioxide 5% and oxygen 95%. The hypogastric nerve was placed on shielded platinum electrodes submerged in the bath at a 1·5 to 2 cm distance from the vas and connected to an electronic stimulator. Rectangular pulses, 100 of 0·5 msec duration, were applied at 2 min intervals, at the alternate frequencies of 10 and 50 shocks/sec; the voltage was supramaximal.

The temperatures quoted in the text are the temperatures, measured directly, of the perfusion fluid in which the organ was submerged.

The following drugs were used: physostigmine (eserine) sulphate, hexamethonium bromide, noradrenaline, isoprenaline hydrochloride, bretylium tosylate, guanethidine sulphate, veratrine, xylocholine bromide, atropine sulphate, mecamylamine hydrochloride, dihydroergotamine methane sulphonate, phenoxybenzamine hydrochloride, phentolamine methane sulphonate, ephedrine sulphate.

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Results

INFLUENCE OF TEMPERATURE ON THE RESPONSES OF THE VAS DEFERENS TO ELECTRICAL STIMULATION

With progressive lowering of bath temperature from 32 to 20°, responses to 50 shocks/sec became gradually smaller, while those to 10 shocks/sec increased (Fig. 1). The phenomenon began in the temperature range

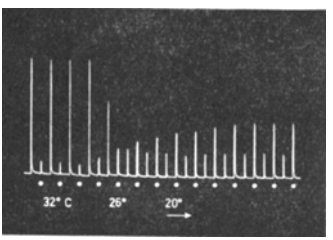

FIG. 1. Contractions of guinea-pig isolated vas deferens in response to hypogastric nerve stimulation. Each stimulation consisted of 100 shocks, applied alternately at the frequency of 50 and 10 shocks/sec (at dots), every 2 min. It may be observed that progressive lowering of bath temperature from 32 to 20° brings about a strong reduction of the responses to the high frequency stimulation, while those to low frequency become markedly increased.

28 to 25°, and became more pronounced the more the temperature was lowered. At 20°, the lowest temperature investigated, the height of contractions reached its minimum and remained constant throughout the experiment. At 20°, the responses to the higher frequency of stimulation appeared to be reduced by 60–80% compared to those at 32°, while the contractions at 10 shocks/sec were increased 5 to 10 fold. A complete and almost immediate reversal of the phenomenon was observed by raising the temperature of the bath to 32° (Fig. 2). In 5 out of 40 preparations

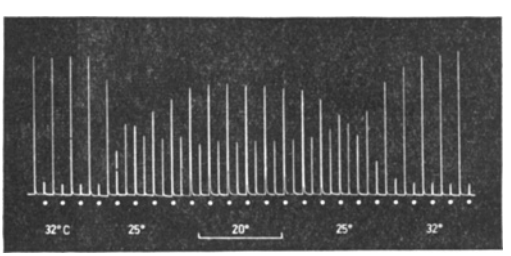

FIG. 2. Same parameters as for Fig. 1. As illustrated in Fig. 1, at 20° the responses to 10 shocks/sec are significantly potentiated and those to 50 shocks/sec greatly reduced. Note the complete reversibility of the effect occurring upon raising the temperature to 32°.

reducing the temperature caused a reduction in the high-frequency responses but did not affect those at low frequency (Fig. 3).

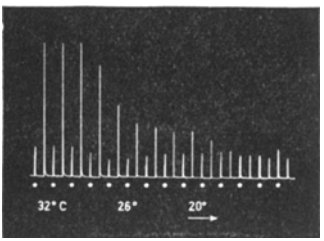

FIG. 3. Same parameters as for Fig. 1. In this preparation lowering of the temperature modifies the responses to the higher frequency of stimulation as previously shown, but leaves those to low frequency unaffected.

INFLUENCE OF TEMPERATURE ON THE RESPONSES OF THE VAS DEFERENS TO DRUGS

Noradrenaline. At 20°, in nearly all experiments the vas deferens was almost unresponsive to direct stimulation with 1–2·5 μg/ml of noradrenaline. The same concentration of noradrenaline caused a marked increase of the responses to hypogastric stimulation at both frequencies, the effect being easily and quickly reversible by washing (Fig. 4).

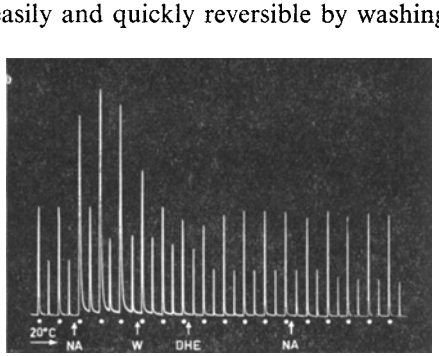

FIG. 4. Same parameters as for Fig. 1. Temperature of the bath, 20°. Addition of noradrenaline to the bath (at NA, 2·5 μg/ml) significantly enhances the responses of the vas to hypogastric stimulation at both frequencies and disappears upon washing (at W). The same dose of noradrenaline, given after dihydroergotamine (at DHE, 1 μg/ml), leaves the height of responses unimpaired.

Adrenergic blocking drugs. Dihydroergotamine, phenoxybenzamine and phentolamine at doses from 0·5 to 10 μg/ml did not reduce the responses of the preparation; in a few instances phenoxybenzamine caused a slight increase (Fig. 5).

In the presence of these drugs, noradrenaline failed to potentiate the responses to stimulation of the hypogastric nerve (Fig. 4). The antagonistic effect did not disappear upon washing out the adrenergic blocking drug; the addition of noradrenaline at this moment sometimes markedly reduced the responses of the preparation to either frequency of stimulation.

Isoprenaline. Concentrations of isoprenaline ranging between 0·25 and 1 μg/ml diminished the responses of the preparation to electrical stimulation. The degree of reduction was reversible upon washing and dependent on the dose used.

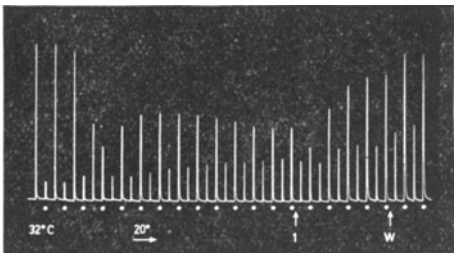

FIG. 5. Same parameters as for Fig. 1. At 20°, treatment with phenoxybenzamine (at 1, 10 μg/ml) enhances the responses of the preparation to the electrical stimulation. At W, washing of the preparation.

Ganglion blocking drugs. Mecamylamine, 5–10 μg/ml, and hexamethonium, 25–50 μg/ml, at 20°, just as at 32° (Sjöstrand, 1962; Birmingham & Wilson, 1963), strongly reduced the responses of the preparation to stimulation of the hypogastric nerve (Fig. 6). The presence of the

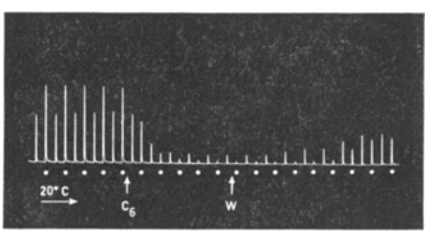

FIG. 6. Same parameters as for Fig. 1. As at 32°, hexamethonium at 20° (at C_6, 50 μg/ml) strongly reduces the responses to both frequencies of stimulation. The effect is more evident for the low-frequency responses. Note the slow reversal of the inhibitory effect of hexamethonium even after washing (at W).

ganglion blocking agent affected the responses at low frequency more than those at high frequency; this was particularly evident for hexamethonium.

Atropine. No modifications of responses occurred upon addition of atropine to the bath at doses from 0·5 to 1 μg/ml.

Physostigmine. At 20°, just as at normal temperature (Boyd, Chang & Rand, 1960; Burn & Weetman, 1963), physostigmine in concentrations ranging from 2 to 5 μg/ml gave rise to a typical enhancement of responses to either frequency of stimulation (Fig. 7). The only difference from the observations at 32° was a longer latency period.

Again as previously described for normal temperature (Della Bella, Benelli & Gandini, 1964), addition of atropine, 0·25–0·50 μg/ml, to a preparation whose responses were potentiated by physostigmine, caused an immediate strong reduction of the responses (Fig. 7).

Veratrine. Veratrine, 0·1–2 μg/ml, produced a reduction, the degree of which appeared related to the dose and which was complete at high concentrations. As with the observations at 32° (Della Bella & Benelli, 1964), no enhancement of responses was observed.

Adrenergic neurone blocking agents. In most of the experiments the responses of the preparation at 20° were reduced by guanethidine at doses

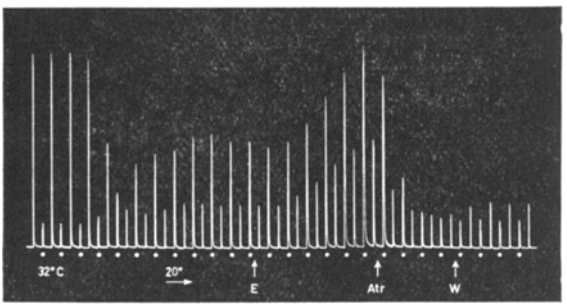

FIG. 7. Same parameters as for Fig. 1. At 20 , physostigmine (at E, 5μg/ml) enhances the responses of the vas to hypogastric stimulation. Addition of atropine (at Atr, 0·5 μg/ml) when the enhancement by physostigmine has developed, causes an immediate reduction in the responses.

of 2·5–10 μg/ml (Fig. 8). But, in three out of nine preparations, guanethidine was completely ineffective. Any reduction seen was less than that observable under the same conditions at 32° and was reversible by 5 μg/ml of ephedrine or 1–2·5 μg/ml of noradrenaline. Pretreatment

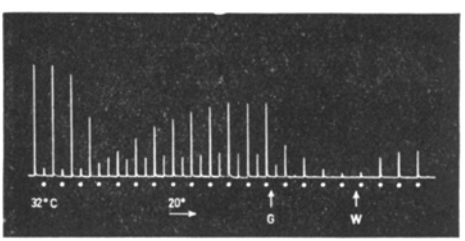

FIG. 8. Same parameters as for Fig. 1. At 20°, addition of guanethidine to the bath (at G, 5 μg/ml) reduces the responses of the preparation to either stimulation frequency strongly. The block appears slowly reversible upon washing (at W).

with the same concentrations of noradrenaline prevented the guanethidine block (Fig. 9). Xylocholine and bretylium partially reduced the responses of the preparation to both stimulation frequencies only at doses 5 to 10

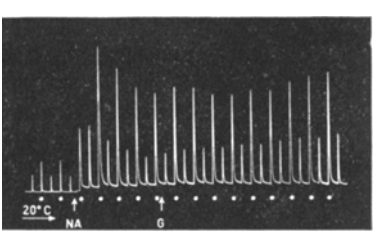

FIG. 9. Same parameters as for Fig. 1. At 20°, addition of noradrenaline to the bath (at NA, 2·5 μg/ml) induces a clear potentiation of the responses to hypogastric stimulation which are unaffected by addition of guanethidine (at G, 5 μg/ml).

times greater than those which blocked at 32°: the loss of activity appeared to be more pronounced for bretylium, which in a few instances potentiated the height of contractions (Fig. 10). Prolonged contact of the preparation

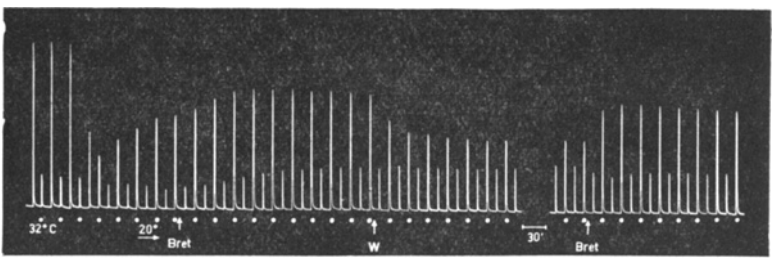

FIG. 10. Same parameters as for Fig. 1. At 20°, unlike at 32°, bretylium (at Bret, 10 μg/ml, then 20 μg/ml) fails to block the responses, which, after the second dose appear potentiated.

with high concentrations of bretylium (20–30 μg/ml) for periods of 30–60 min, did not affect the responsiveness of the vas which, when returned to 32°, responded as usual to hypogastric stimulation.

Discussion

In line with previous findings on a variety of preparations *in vivo* and *in vitro* (Brown, 1954; Malméjac, Neverre & Malméjac, 1956; Kosterlitz & Robinson, 1957; Maclagan & Zaimis, 1957; Mainwood, 1957; Li, 1958; Young, 1959; Della Bella, Gandini & Teotino, 1963), the observations made by us on the guinea-pig isolated hypogastric nerve-vas deferens preparation suggest a pronounced influence of temperature on processes connected with both ganglionic and neuromuscular transmission.

On decreasing the bath temperature from 32 to 20°, the responses to 50 shocks/sec became progressively smaller, being reduced by some 60 to 80% at 20°, and those to 10 shocks/sec were increased 5 to 10 times the initial values.

How the change in temperature produced these changes may be explained in the light of the results obtained by Kuriyama (1964) who used a microelectrode technique in his study of neuromuscular transmission in the vas deferens at different temperatures. At low temperature this worker found the rising and falling phases of junction potentials to be of relatively smaller amplitude but of much longer duration than those at higher temperature. If we consider that a longer refractory period was a consequence of these potential modifications, which in the preparation we examined occur probably at two different levels—ganglionic and neuromuscular, it seems likely that at low temperature the higher frequency of stimulation was insufficient to allow transmission, so that most stimuli were ineffective, while the lower frequency stimuli became of optimal frequency so that all stimuli were effective.

The finding that the responses at low frequency appear higher at 20° than at 32°, may be explained in the light of the following suggestions.

Firstly, if the temperature reduction acted directly on the contractile mechanism to greatly slow its operation rate, though to reduce only moderately the total amount of shortening (Keatinge, 1964), at 20° and at the frequency of 10 shocks/sec there now existed an optimal summation of the single contractions elicited by successive stimulations. It is well known that on *striated* muscle the frequency of stimulation necessary to produce tetanic fusion is markedly decreased by cooling (Maclagan & Zaimis, 1957). In addition, as suggested by Bigland, Goetzee, Maclagan & Zaimis (1958) to explain the relative insensitivity of *striated* muscle to (+)-tubocurarine at low temperature, we cannot exclude that at the lower temperature when there was enhanced height of the vas deferens contractions, there was also a slower diffusion rate of the chemical transmitter from both ganglionic and neuromuscular synaptic spaces. It is known that elimination of chemical mediator from these sites occurs either through enzymatic reactions or by simple diffusion in the medium, and both these processes are shown to possess a high temperature coefficient (Eccles & Jaeger, 1958).

Experiments now in progress in our laboratories, aiming to test the influence of temperature on the onset of direct responses of the vas deferens to noradrenaline, seem to favour this hypothesis: while at 30° noradrenaline elicits immediate responses, at low temperature the onset of contractions is characterised by a latency of 45–50 sec. In our opinion there are grounds for thinking that such a latency period may be due to the reduced diffusion rate of the drug and to delay in its reaching the action site: there would then be a phenomenon analogous to that described above, but the diffusion would be occurring in the opposite direction.

Another mechanism involved in neuromuscular and ganglionic transmission which could be affected by temperature changes, is the synthesis of chemical mediators. We know of no experiments made in this connection on noradrenaline; but in 1956, Kostial & Vouk investigated the effect of temperature on the acetylcholine output after preganglionic stimulation of the cat superior cervical ganglion at 2 shocks/sec. These authors, unlike Brown (1954) who used a frequency of 10 shocks/sec, did not find any variation for temperature values from 39 to 20°. Kostial & Vouk concluded that the slower reaction rate of the choline-acetylating process, which occurred at low temperature, and the resulting shortage of chemical mediator, were likely to be the main causes of the reduced effectiveness of the stimuli at 10 shocks/sec. This hypothesis was later substantiated by results obtained *in vitro* on the enzyme from mammalian brain (Milton, 1958).

Nevertheless, on the neuromuscular synapses of the rat vagus nerve-stomach isolated preparation, we failed to demonstrate an actual diminution of the stores of chemical mediator at low temperature. Parallel to the decrease of temperature from 32 to 20°, responses to electrical stimulation showed a progressive reduction which became a complete disappearance of motor responses. This was not seen with responses to dimethylphenylpiperazinium (DMPP) the stimulating activity of which is exerted through excitation of the ganglionic cell and the subsequent

liberation of acetylcholine at postganglionic nerve endings (Della Bella & others, 1963). Analogous results were obtained by Gillespie & Wishart (1957) in experiments directed to testing the effect of cooling on the responses of the rabbit colon to nerve and to drug stimulation.

If we now examine the behaviour of the vas deferens at 20° under drug treatment, we conclude that the preparation responds to noradrenaline, isoprenaline, adrenergic blocking agents, atropine, physostigmine, veratrine and ganglion blocking agents almost as it did at 32°.

About the effects of noradrenaline, two observations on the preparation pretreated with adrenergic blocking agents are worth stressing. The first concerns the partial and promptly reversible block of responses to noradrenaline in such conditions: a likely explanation for this inhibitory effect could be an indirect ganglion blocking activity exerted through a reduction of the cholinergic mediator output at preganglionic neurone terminals, as demonstrated by Lundberg (1952) on the superior cervical ganglion. The second relates to the complex question of the adrenergic nature of the nervous transmission mechanism at the neuromuscular junction of the vas deferens. It is known that adrenergic blocking agents are more effective against added noradrenaline than against the endogenously released amine, which is generally supposed to be responsible for the responses of the vas to hypogastric stimulation. If the enhancement by noradrenaline of the responses to electrical stimulation observed by us at 20° is to be regarded as subsequent to its uptake into storage sites and to its increased availability, as already suggested by Huković (1961) for the reserpinised preparation, we may make three postulates. These are that the inhibitory effect of adrenergic blocking agents towards such an enhancement may be due either to: (1) a selective antagonism towards the noradrenaline liberated by electrical stimulation from an easily releasable and reconstitutable pool—it is possible that several pools exist (Kuntzman & Jacobson, 1964; Potter, Axelrod & Kopin, 1962; Trendelenburg, 1961), or (2) to a prevention of the uptake of the transmitter at the storage site (Farrant, Harvey & Pennefather, 1964) or (3) to a combination of these mechanisms.

Another interesting result emerging from our study is the reduced adrenergic neurone blocking activity demonstrated at low temperatures by xylocholine and bretylium in particular. The finding, not very different from the observations on (+)-tubocurarine at *striated* neuromuscular synapses (Holmes, Jenden & Taylor, 1951) appears difficult to explain. The fact that after contact with bretylium at 20° the preparation again exhibits a normal responsiveness to the drug upon raising the temperature of the bath to 32°, leads us to suppose that at 20° the drug does not link up as usual with its action site. The failure of the drug to combine with the receptor seems rather surprising, since bretylium is known to be specifically taken up and securely bound by adrenergic fibres (Boura, Copp, Duncombe, Green & McCoubrey, 1960). A possible explanation may be advanced either on the basis of a reduced diffusion and a consequent accumulation of a hypothetical chemical mediator with which bretylium may compete, this by analogy with what is postulated for

(+)-tubocurarine by Bigland & others (1958), or, in line with the suggestions of Holmes & others (1951), it may be that there is some effect of temperature on the drug diffusion process and in particular on the reaction rate and the equilibrium conditions between the drug itself and its receptor.

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