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GUANETHIDINE AND GUANACLINE ON THE RAT VAS DEFERENS

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1 In acute experiments guanethidine was considerably more potent than guanacline in reducing contractile responses of the rat vas deferens to electrical stimulation of intramural nerves.

2 Chronic treatment of rats for 19 weeks with guanethidine (5mg/kg, daily) reduced responses to electrical stimulation to 25% of control, potentiated responses to exogenous noradrenaline and depleted endogenous noradrenaline.

3 After cessation of guanethidine treatment responses to electrical stimulation increased to 60% of control (in one week) but showed no further increase. There was no decrease in the potentiation of exogenous noradrenaline (after 14 weeks) nor any increase in endogenous noradrenaline levels (after 7 weeks).

4 Chronic treatment of rats for 19 weeks with guanacline (5mg/kg, daily) potentiated responses to exogenous noradrenaline and depleted endogenous noradrenaline as much as guanethidine treatment but did not reduce responses to electrical stimulation.

5 On cessation of guanacline treatment there was some increase in noradrenaline content (after 2 to 3 weeks) and some decrease of potentiated responses to exogenous noradrenaline (after 2 weeks).

6 The noradrenaline-depleting action of these drugs is distinct from blockade of nerve-mediated responses in the rat vas deferens and contractile function after guanethidine treatment can be partly restored despite persistence of noradrenaline depletion and supersensitivity.

Introduction

In a previous study (Gannon, Iwayama, Burnstock, Gerkens & Mashford, 1971) chronic treatment of rats for up to 18 weeks with guanethidine or guanacline markedly reduced the neuronal catecholamine fluorescence of the vas deferens. Moreover, after cessation of guanethidine treatment the catecholamine fluorescence of nerve fibres in the muscle layers of the vas deferens showed no reappearance even after 6 months. It was suggested that chronic treatment with guanethidine may produce long-term damage to the adrenergic innervation of the vas deferens.

This study describes the effect, on the function of the rat vas deferens, of the acute addition of guanethidine or guanacline to isolated vasa and of the chronic treatment of rats with guanethidine or guanacline.

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Methods

Two groups of 40 sexually isolated male rats (175-310g) were used for chronic treatment. One group received daily intraperitoneal injections of guanacline sulphate (Bayer; 5mg/kg) and the other guanethidine sulphate (Ciba; 5mg/kg). The drug solutions, in 0.9% w/v NaCl solution (saline), were made from the solid compound shortly before injection. Injection volume was 1 ml/kg. Three rats from each drug-treated group were used for experiments after 4 weeks treatment. The remainder were treated for 19 weeks.

Experiments were performed on rats from each drug-treated group 4 weeks and 19 weeks after the start of treatment and at 1, 2, 3, 5, 7, 8 and 14 weeks after cessation of treatment. Animals were anaesthetized with ether, pithed and used for cardiovascular tests before removal of both vasa deferentia. Untreated rats were used as controls.

One vas deferens was placed in ice cold saline,

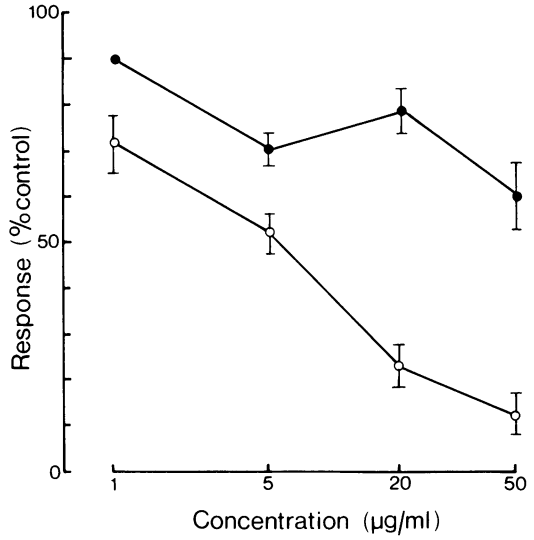

Fig. 1 Relation of reduction in contractile responses of isolated rat vasa deferentia to concentration of guanethidine (○) or guanacline (●) in acute experiments. Stimulation at 5 Hz for 10 s every 4 min with 1 ms pulses. Responses 30 min after addition of drug are expressed as a percentage of pre-drug responses. Each point represents the mean result obtained with 3 or 4 preparations. Vertical bars are s.e. mean. Guanethidine is much more potent than guanacline.

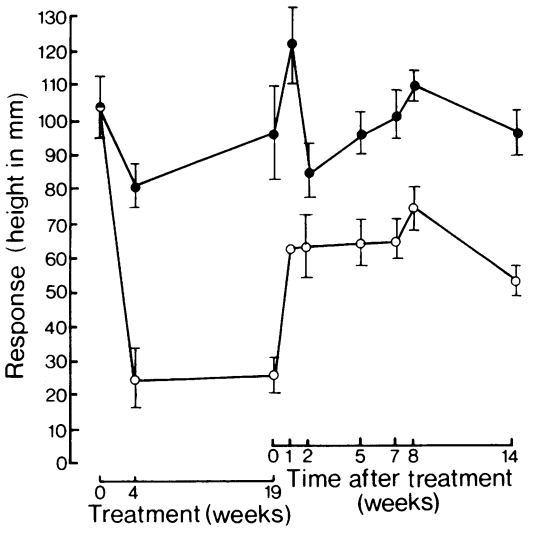

Fig. 2 Effect of 4 and 19 weeks treatment (5 mg/kg, daily) with guanethidine (○) or guanacline (●), and of periods up to 14 weeks after cessation of treatment, on the contractile response of the isolated vas deferens to electrical stimulation (as in Figure 1). During guanethidine treatment responses were markedly reduced whereas guanacline treatment produced no significant reduction. After cessation of guanethidine treatment there was partial recovery. Each point represents the mean result obtained from 3 to 5 preparations. Vertical bars are s.e. mean.

patted dry and transferred to a pre-weighed bottle containing 5 ml ice-cold 0.4 M perchloric acid. The noradrenaline content was assayed according to the method of Anton & Sayre, (1962).

The second vas deferens from each animal was set up in a 50 ml organ bath containing McEwen's (1956) solution (NaCl 7.6, KCl 0.42, $CaCl_2$ 0.24, NaH_2PO_4 0.143, $NaHCO_3$ 2.1, dextrose 2.0, sucrose 4.5, g/litre) at 37°C and continually aerated with 95% oxygen and 5% carbon dioxide. Contractions were recorded by an isotonic lever (magnification six fold, tension 0.35 g) writing on a smoked kymograph paper. The length of the vas deferens in the organ bath was 40-45 mm.

The intramural nerves were electrically stimulated, by means of a bipolar platinum ring electrode around the prostatic end of the vas deferens, with 1 ms pulses of supramaximal voltage at 5 Hz for 10 s every 4 minutes. Contractions to 0.2 and 1.0 μg/ml noradrenaline bitartrate were also obtained.

In the acute experiments preparations from untreated rats were stimulated as above. Guanethidine or guanacline was added to the organ bath and left for 30 minutes.

Means were compared by Student's *t* test, $P < 0.05$ being accepted as significant.

Results

Nerve stimulation

In acute experiments guanacline was considerably less potent than guanethidine (Figure 1). At the highest dose used (50 μg/ml) guanethidine almost abolished the response to stimulation whereas guanacline reduced the responses by only 40% (s.e. ±7%).

In rats treated chronically with guanethidine for 4 and 19 weeks the response of the vas deferens to electrical stimulation was reduced to 25% of control ($P < 0.01$; Figure 2). In contrast, guanacline treatment at no time significantly affected responses to nerve stimulation.

After cessation of guanethidine treatment the responses increased to, and remained at, about 60% of control. At all times up to 14 weeks after cessation of treatment the responses were less than control values ($P < 0.05$) but greater than responses obtained at the end of guanethidine treatment ($P < 0.05$).

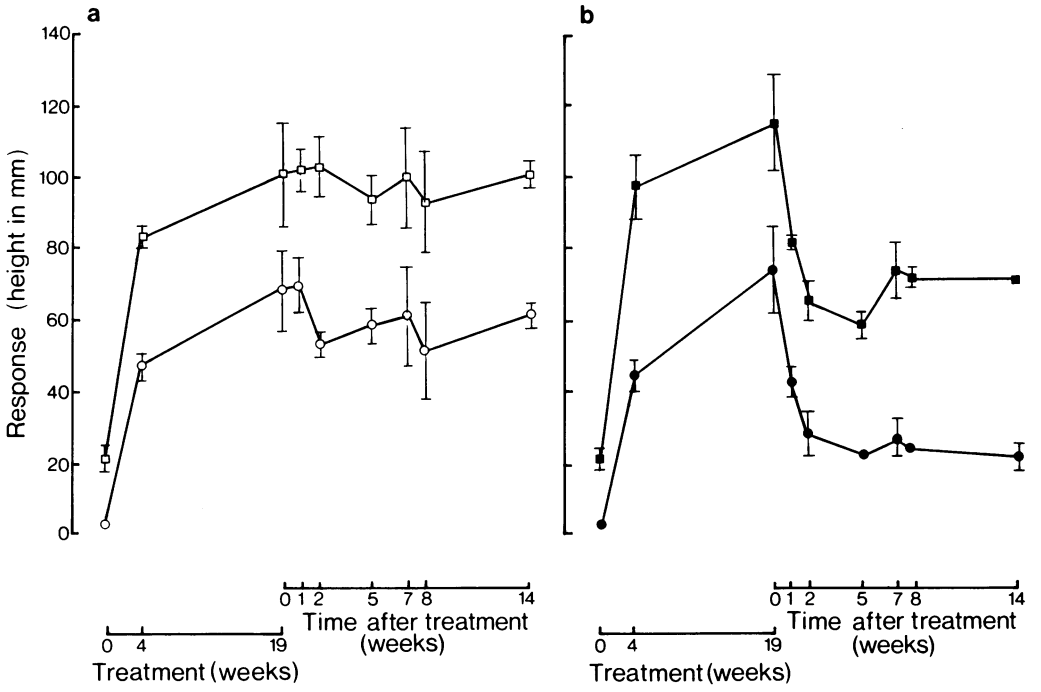

Fig. 3 Effect of 4 and 19 weeks treatment (5 mg/kg daily) with (a) guanethidine or (b) guanacline, and of periods up to 14 weeks after cessation of treatment, on the contractile response of the isolated vas deferens to noradrenaline 0.2 μg/ml (○,●) and 1.0 μg/ml (□,■). Both treatments produced a marked and similar potentiation of noradrenaline responses and this potentiation persisted unchanged after cessation of guanethidine treatment. Each point represents the mean result obtained with from 3 to 5 preparations. Vertical bars are s.e. mean.

Exogenous noradrenaline responses

Nineteen weeks treatment with either guanethidine or guanacline produced a marked potentiation ($P < 0.01$) of responses to exogenous noradrenaline (Figure 3). There was no difference at this time between the potentiation produced by the two treatments. On cessation of guanethidine treatment the potentiation was maintained, showing no change after 14 weeks. On the other hand, after cessation of guanacline treatment the potentiation of noradrenaline responses showed a limited decrease. From 2 to 14 weeks after cessation of guanacline treatment the responses were less than at the end of 19 weeks treatment ($P < 0.01$) but still greater than controls ($P < 0.01$).

Endogenous noradrenaline content

The noradrenaline content of the vas deferens was reduced by guanethidine treatment to 30% ($P < 0.05$) and by guanacline treatment to 25% ($P < 0.01$) of control values (Figure 4). The difference

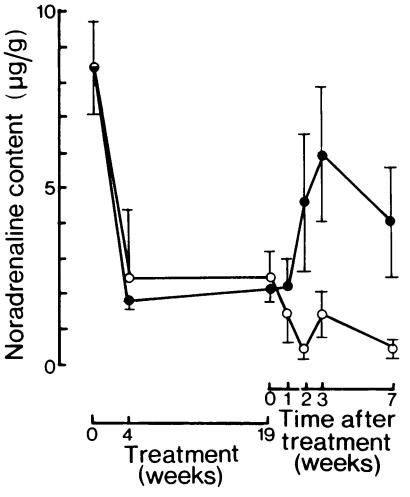

Fig. 4 Effect of 4 and 19 weeks treatment (5 mg/kg daily) with guanethidine (○) or guanacline (●), and of periods up to 7 weeks after cessation of treatment, on the noradrenaline content of the vas deferens. Both treatments produced a similar depletion of noradrenaline content and there was no recovery from this depletion after cessation of guanethidine treatment. Each point represents the mean result obtained with from 3 to 5 preparations. Vertical bars are s.e. mean.

between the depletion produced by the two drugs was not significant. Up to 7 weeks after cessation of guanethidine treatment there was no increase in the noradrenaline content. After cessation of guanacline treatment there was a partial recovery of noradrenaline levels. This was significant ($P < 0.05$) when the values after 2 and 3 weeks were pooled.

Discussion

Chronic treatment of rats with either guanethidine or guanacline in this study produced a very similar depletion of noradrenaline stores of the vas deferens. On the other hand, guanethidine treatment markedly reduced responses to electrical stimulation (to 25% of the control level) whereas guanacline treatment did not produce any significant reduction of these responses. Furthermore, after cessation of guanethidine treatment the previously-blocked responses to electrical stimulation recovered to about 60% of the control level. This recovery to stimulation however was not matched by any recovery of the depleted noradrenaline stores.

There is much evidence both pharmacological and electrophysiological which indicates that noradrenaline is the motor transmitter in the vas deferens (Birmingham & Wilson, 1963; Burnstock & Holman, 1964). However, it has been shown that normal or near-normal responses to electrical stimulation in sympathetically innervated tissues do occur despite extensive noradrenaline depletion. Thus Fielden & Green (1967) showed that after treatment with (−)-β-hydroxyphenethylguanidine the response of the cat nictitating membrane to stimulation was little affected despite the loss of more than 90% of the noradrenaline content. Recently, Wakade & Krusz (1972) also found in the guinea-pig vas deferens, that in spite of a 99% reduction in noradrenaline content after reserpine-treatment, the contractile response to stimulation was still about 60% of normal. It is possible that in the present study, the normal responses to electrical stimulation obtained during guanacline treatment, despite noradrenaline depletion, are due to the release of enough noradrenaline to stimulate the adrenergic receptors sufficiently to cause a normal contraction. That such contractile responses could be obtained with even a reduced release of noradrenaline is supported by the fact that during guanacline treatment the vas deferens is super-sensitive to noradrenaline (Figure 3). This supersensitivity is most probably due to a guanacline-induced block of the noradrenaline uptake process (Schümann & Philippu, 1968).

In contrast to guanacline, which does not reduce responses, the block of responses to electrical stimulation produced during guanethidine treatment could be due to the potent and specific adrenergic neurone blocking action of this drug, independent of noradrenaline depletion. This action has been well documented and reviewed (Boura & Green, 1965; Fielden & Green, 1967) and would result in a much-reduced release of noradrenaline. Although there are no published reports comparing the adrenergic neurone blocking potency of guanethidine and guanacline, the relative effect of these two drugs in acute experiments would indicate that guanacline was a much weaker adrenergic neurone blocking drug, that is, it would not block the release of noradrenaline as much as would guanethidine. Therefore the fact that guanacline-induced depletion during chronic treatment does not reduce stimulation responses in the vas deferens is probably a consequence of its much weaker adrenergic neurone blocking action, the release of some noradrenaline and the supersensitive state of the tissue. It is still consistent with an adrenergic innervation but might support the suggestion, by Ambache & Zar (1971), that the motor response may be at least partly non-adrenergic.

The persistent depletion of noradrenaline levels and supersensitivity to exogenous noradrenaline after cessation of guanethidine treatment are similar to the effects of denervation in the cat nictitating membrane (Smith, Trendelenburg, Langer & Tsai, 1966). However, chronic treatment of rats with guanethidine (10 mg/kg) for 13 weeks has recently been shown not to decrease the number of axons nor to produce any degenerating axons in the vas deferens (Evans, Iwayama & Burnstock, 1973). Although these authors reported no restoration, after cessation of treatment, of contractile responses to stimulation previously blocked by guanethidine treatment, Hepp & Kreye (1973) did find that ejaculatory function was restored 8 weeks after guanethidine treatment had finished (25 mg/kg daily for 8 weeks). The latter result agrees with the findings in the present study where contractile responses were partly restored after cessation of guanethidine treatment. It seems that the nerves are not destroyed by guanethidine treatment and are able to synthesize and release noradrenaline after cessation of treatment, but are not able to take up and store noradrenaline to the same extent. At this stage, the ability to release more noradrenaline than during treatment, and the partial recovery of responses to electrical stimulation could be due to the absence, when the drug is no longer present, of the adrenergic neurone blocking action of guanethidine. The effect of the transmitter released, albeit reduced in amount compared to normal because of the depleted state,

would be potentiated because of the persistent supersensitivity to noradrenaline.

A persistent impairment of the membrane noradrenaline uptake mechanism would account for the persistent depletion of noradrenaline and super-sensitivity to exogenous noradrenaline. The effect of guanacline treatment appears to be similar but less marked since after cessation of treatment there is a partial return to normal of both noradrenaline content and responses to exogenous noradrenaline.

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References

AMBACHE, N. & ZAR, M.A. (1971). Evidence against adrenergic motor transmission in the guinea-pig vas deferens. *J. Physiol., Lond.*, **216**, 359-389.

ANTON, A.H. & SAYRE, D.F. (1962). A Study of the factors affecting the aluminium oxide-trihydroxyindole procedure for the analysis of catecholamines. *J. Pharmac. exp. Ther.*, **138**, 360-375.

BIRMINGHAM, A.T. & WILSON, A.B. (1963). Preganglionic and postganglionic stimulation of the guinea-pig isolated vas deferens preparation. *Br. J. Pharmac. Chemother.*, **21**, 569-580.

BOURA, A.L.A. & GREEN, A.F. (1965). Adrenergic neurone blocking agents. *A. Rev. Pharmac.*, **5**, 183-212.

BURNSTOCK, G. & HOLMAN, M.E. (1964). An electrophysiological investigation of the actions of some autonomic blocking drugs on transmission in the guinea-pig vas deferens. *Br. J. Pharmac. Chemother.*, **23**, 600-612.

EVANS, B., IWAYAMA, T. & BURNSTOCK, G. (1973). Long-lasting supersensitivity of the rat vas deferens to norepinephrine after chronic guanethidine administration. *J. Pharmac. exp. Ther.*, **185**, 60-69.

FIELDEN, R. & GREEN, A.L. (1967). A comparative study of the noradrenaline-depleting and sympathetic-blocking actions of guanethidine and (−)-β-hydroxyphenethylguanidine. *Br. J. Pharmac. Chemother.*, **30**, 155-165.

GANNON, B.J., IWAYAMA, T., BURNSTOCK, G., GERKENS, J. & MASHFORD, M.L. (1971). Prolonged effects of chronic guanethidine treatment on the sympathetic innervation of the genitalia of male rats. *Med. J. Aust.*, **2**, 207-208.

HEPP, R. & KREYE, V.A.W. (1973). Effect of long-term treatment with high doses of guanethidine on sperm transport and fertility of rats. *Br. J. Pharmac.*, **48**, 30-35.

McEWEN, L.M. (1956). The effect on the isolated rabbit heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain. *J. Physiol., Lond.*, **131**, 678-689.

SCHÜMANN, H.J. & PHILIPPU, A. (1968). Die Wirkung von Cyclazenin auf die Speicherung und Freisetzung sympathicomimetischer Amine in Herzen, *Arzneim. Forsch.*, **18**, 1571-1574.

SMITH, C.B., TRENDELENBURG, U., LANGER, S.Z. & TSAI, T.H. (1966). The relation of retention of norepinephrine-H^3 to the norepinephrine content of the nictitating membrane of the spinal cat during development of denervation supersensitivity. *J. Pharmac. exp. Ther.*, **151**, 87-94.

WAKADE, A.R. & KRUSZ, J. (1972). Effect of reserpine, phenoxybenzamine and cocaine on neuromuscular transmission in the vas deferens of the guinea pig. *J. Pharmac. exp. Ther.*, **181**, 310-317.

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