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Persistent neuronal blockade with guanethidine in dog mesenteric arteries

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The effects of prolonged administration of guanethidine differs in several respects from those following acute dosage (Boura & Green, 1965; Laverty, 1973; Jandhyala, Clarke & Buckley, 1974). We have shown (Clarke, Jandhyala & others, 1974) that tolerance develops to the adrenergic neuronal blocking action of guanethidine to the dog heart, whereas sympathetic activity to the mesenteric arteries remains inhibited. Thus, the present study was undertaken to define further the nature and time-course of guanethidine-induced effects upon neuronal and receptor function in the mesenteric arteries of the dog. The findings show that guanethidine produces rapid but long-lasting effects which appear to be only slowly reversible with time. Additionally, persistent neuronal blockade is associated with a resistance to (+)-amphetamine-induced reversal.

Purebred beagle dogs were treated daily with oral guanethidine (2·5 mg kg^{-1}) for various times. Controls, randomly selected, received lactose. The guanethidine or lactose in a gelatin capsule was placed at the back of a dog's tongue, swallowing was initiated by neck massage. All experiments were made 18 to 22 h after the last dose. Isolated mesenteric artery preparations were obtained after the administration of sodium pentobarbitone (35 mg kg^{-1}, i.v.) as described previously (Clarke, Ertel & others, 1972). The vessels were perfused with Krebs bicarbonate solution at a constant rate (35 ml min^{-1}) and perfusion pressure recorded. At least three preparations were made from each dog and the results were pooled to give a single "n" value. The periarterial sympathetic nerves were stimulated at a supramaximal voltage, with a pulse duration of 2 ms over a range of frequencies (1–24 Hz) for 20 s at 3 to 6 min intervals. Drugs were injected into the perfusion fluid.

Fig. 1 shows the effect of treatment with guanethidine for 1 and 7 days. After only one dose of guanethidine the responses to injected noradrenaline were increased by about two-fold, but the frequency-response curve did not differ from the lactose controls. As there was increased sensitivity to injected noradrenaline some impairment of neuronal function was already present. After 7 days of treatment, neurotransmission was fully inhibited but there was no further increase in the responses to injected noradrenaline. Treatment for 2 and 8 months (Fig. 2) also failed to increase further the sensitivity to injected noradrenaline. Neuronal blockade persisted, confirming data presented previously for 6 months of treatment (Clarke, Jandhyala & others, 1974). In dogs given guanethidine for 6 months, followed by lactose administration for 2 months, the sensitivity to noradrenaline returned to within control values, but nerve mediated responses remained clearly less than those obtained in the control preparations.

It is well established that (+)-amphetamine will reverse the early sympathetic neuronal blocking action of guanethidine (Day & Rand, 1962, 1963; Boura & Green, 1965). In the current study (+)-amphetamine (4 mg, bolus dose) readily reversed the effects of guanethidine after 1 or 7 days of treatment. However,

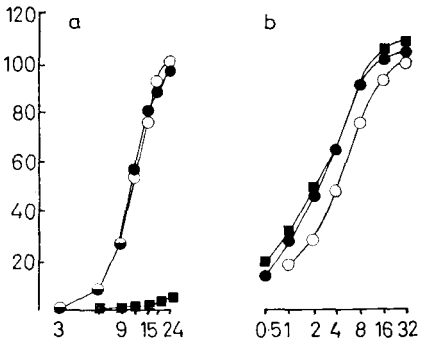

FIG. 1. Effect of guanethidine (2·5 mg kg^{-1} day^{-1}, orally) on the frequency response to (abscissae) a-periarterial nerve stimulation (Hz) and b-injected noradrenaline (μg) in isolated perfused dog mesenteric arteries. Pooled controls, lactose for 1 and 7 days (n = 6), ○—○; guanethidine for 1 day (n = 3), ●—●; guanethidine for 7 days (n = 3), ■—■. The maximum control rise in perfusion pressure (%) (ordinate) for nerve stimulation and noradrenaline was 210 ± 18 and 218 ± 12 (mm Hg, mean ± s.e.m.), respectively. Significance of differences ($P<0·05$): nerve stimulation, ■—■; noradrenaline, ■—■, ●—● (between 20 and 80% values).

* Correspondence.

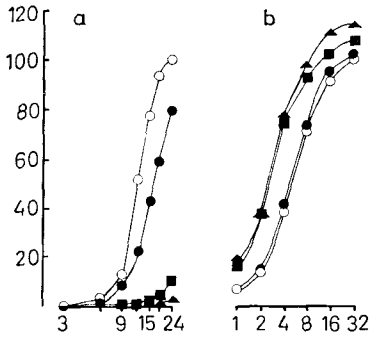

FIG. 2. As for Fig. 1. Control, lactose for 8 months (n = 4), ○—○; guanethidine for 6 months then lactose for 2 months (n = 4), ●—●; guanethidine for 8 months (n = 4), ■—■; lactose for 6 months then guanethidine for 2 months (n =), ▲—▲. The maximum control (lactose for 8 months) rise in perfusion pressure for nerve stimulation and noradrenaline was 248 ± 20 and 244 ± 18, (mm Hg, mean ± s.e.m.), respectively. Significance of differences from respective controls ($P<0.05$): nerve stimulation, ▲—▲, ■—■, ●—● (between 12 and 24 Hz); noradrenaline, ▲—▲, ■—■.

after 8 months of treatment the drug failed to restore periarterial nerve function, even after repeated administration.

The present data describe both a rapid and persistent susceptability of the dog mesenteric sympathetic innervation to oral guanethidine. The daily dose (2·5 mg kg^{-1}) is by no means excessive since it approximates to the upper limit of the recommended therapeutic dose range for man (Physicians' desk reference, 1973). Higher doses of guanethidine given chronically to rats have revealed clear evidence of neurotoxicity (Jensen-Holm & Juul, 1971; Burnstock, Evans & others, 1971; Gannon, Iwayama & others, 1971; Juul & McIsaac, 1973, Evans, Iwayama & Burnstock, 1973). It is possible, therefore, that a comparatively lower dose in dogs might produce similar effects on the postganglionic sympathetic fibres to the mesenteric vasculature. This contention may explain the increased resistance to (+)-amphetamine-induced reversal with time and also the apparent long-lasting effects of guanethidine which were still evident two months after the cessation of treatment. The fact that tolerance occurs to the neuronal blocking action of guanethidine in the heart (Clarke & others, 1974) implies that there is a critical physiological difference between neurotransmitter mechanisms in the heart from those in the mesenteric arteries. Studies in rats have also shown differential effects. For instance, the sympathetic innervation to the genital organs is preferentially affected by guanethidine compared with that to the heart (Burnstock & others, 1971; Gannon & others, 1971; Evans & others, 1973). Apparently, guanethidine-induced toxicity is also species selective. A recent publication by Johnson, Macia & Yellin (1977) showed that guanethidine was without marked effects on tissue noradrenaline concentration (heart and spleen) and tyrosine hydroxylase activity (superior cervical ganglion) of cats, rabbits and hamsters.

Further evidence for the susceptibility of the dog periarterial nerves to guanethidine may be deduced from the increased responses to noradrenaline since the two-fold increase most probably reflects inhibition of neuronal uptake. A similar degree of potentiation can be obtained by injecting guanethidine (1 to 2 mg) or desipramine (0·5 mg) into the perfusion fluid of control preparations. The lack of an increasing sensitivity to noradrenaline with time may be related to the continued function of adrenal adrenaline release (Boura & Green, 1963; Clarke & Romanyshyn, 1976). Tonic receptor activation by circulating adrenaline would obviate the development of postsynaptic supersensitivity (Trendelenburg, 1963; Fleming 1971).

The presently reported data are consistent with most clinical observations. Studies indicate that the chronic antihypertensive effect of guanethidine is due largely to a reduction in peripheral resistance (Chamberlain & Howard, 1964; Villarreal, Exaire & others, 1964; Sannerstedt & Conway, 1970) and with the exception of one major study (Stocks & Robertson, 1967), it is generally agreed that tolerance to this effect occurs rarely, or not at all (Dollery, Emslie-Smith & Milne, 1961; Bauer, Croll & others, 1961).

October 24, 1977

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5-Methoxy-*NN*-dimethyltryptamine: differential modulation of the rewarding and aversive components of lateral hypothalamic self-stimulation

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It is well established that the catecholamines noradrenaline and dopamine exert a significant excitatory role in self-stimulation behaviour (German & Bowden, 1974). At the same time, 5-hydroxytryptamine (5-HT) has traditionally been seen as having a complementary inhibitory role. The inhibitory role of 5-HT is supported by the widely cited report that *p*-chlorophenylalanine (*p*CPA), a 5-HT synthesis inhibitor, increased the rate of medial forebrain bundle self-stimulation (Poschel & Ninteman, 1971). However, in contrast to the facilitation reported by Poschel & Ninteman (1971) there are also data showing that *p*CPA has no effect on medial forebrain bundle self-stimulation and that it produces an inhibitory effect on raphé or caudate-putamen self-stimulation (Miliaressis, Bouchard & Jacobowitz, 1975; Phillips, Carter & Fibiger, 1976). Interpretation of such widely divergent results is complicated by a number of factors, not the least of which is the complex sequence of monoamine depletion produced by *p*CPA. To further explicate the role of 5-HT in self-stimulation it would seem advisable to explore the effects of drugs that have a more selective effect on 5-HT availability.

Recent studies have shown that the selective 5-HT reuptake blockers LU 10-171 (1-[3-(dimethylamino) propyl]-1-(*p*-fluorophenyl)-5-phthalancarbonitrile) and fluoxetine have an inhibitory effect on lateral hypothalamic self-stimulation (Atrens, Ungerstedt & Ljungberg, 1977; Katz & Carroll, 1977). Atrens & others (1977) showed that this effect was specific to the rewarding component of intracranial stimulation (ICS) and could be clearly dissociated from any non-specific behavioural inhibition. In addition they showed that the specific blockade of noradrenaline reuptake with LU 5-003 produced a similar reduction in self-stimulation reward. They suggested that any pharmacological agent that increased availability of noradrenaline or 5-HT in a response independent manner should attenuate self-stimulation. Using an entirely different paradigm, Franklin & Herberg (1977) arrived at a similar conclusion.

In the present experiment the effects on self-stimulation of 5-methoxy-*NN*-dimethyltryptamine (5-MeODMT), a hallucinogenic indolealkylamine believed to be an agonist at both pre- and postsynaptic 5-HT receptors (Aghajanian & Haigler, 1975) were studied. The use of a two-way shuttle box permitted the differentiation of specific reward and aversion modulation effects from non-specific performance changes.

Eight male Wistar rats, 250–300 g, received stereotaxic implants on 254 μm monopolar stainless-steel electrodes insulated except for the flat cross-section at the tips. The reference electrode was attached to a screw on the skull. Lateral hypothalamic co-ordinates relative to bregma with the skull in a flat position were 2·5 mm posterior, 1·7 mm lateral and 8·7 mm ventral. After postoperative recovery, the rats were tested for self-stimulation in a shuttle box apparatus described previously (Atrens & Becker, 1975; Hunt, Atrens, & others, 1976; Atrens & others, 1977). The ICS consisted of 50 Hz biphasic square wave pulses of 200 μs duration with an anodal pulse immediately following each cathodal pulse. Electronic programming and recording equipment recorded the latencies to initiate and escape ICS which are respectively indices of

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