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An assessment of the cardiovascular sympathectomy induced by guanethidine

T. H. BLYTHE, R. C. HALL* AND I. E. HUGHES

*Department of Pharmacology, The University of Western Australia, Nedlands, Western Australia, 6009, *Department of Human Physiology and Pharmacology, The University of Adelaide, Adelaide, South Australia, 5000*

Guanethidine treatment of rats (30 mg kg^{-1}, i.p. daily for 6 weeks) produced a profound reduction in the catecholamine present (as indicated by fluorescence histochemistry and catecholamine determinations) in tissues taken from the cardiovascular system, but there was evidence of the return of catecholamines within 8 weeks. While these changes are consistent with a sympathectomy, the unaltered pressor responses to physostigmine (100 μg kg^{-1}, i.v.) and to carotid occlusion indicate an unimpaired functional capacity of noradrenergic nerves supplying the cardiovascular system. Although part of the response may be attributed to the unaffected adrenal medulla enhanced by the presence of considerable supersensitivity as shown to exogenous noradrenaline, there would appear to be a dissociation between the results obtained from physical and functional tests of the sympathectomy induced by guanethidine.

A long lasting destruction of noradrenergic nerves supplying the cardiovascular and reproductive systems of rats has been reported following chronic administration of high doses of guanethidine (Burnstock, Evans & others, 1971). Although this report is based on histological, fluorescence histochemical and electron microscopical evidence, other workers (Evans, Iwayama & Burnstock, 1973; Gerkens, 1974) have confirmed that in the reproductive and cardiovascular systems this destruction is associated with an impairment of the functional capacity of the neurons. However, in the cardiovascular system the impairment was incomplete and this may have been due to the small doses of guanethidine used. It seemed worthwhile, therefore, to re-examine in functional terms the cardiovascular sympathectomy induced by the higher dose of guanethidine used by Burnstock & others (1971) because such a simple technique for inducing a long lasting sympathectomy would be a useful tool in evaluating the role of noradrenergic nerves in, for example, experimental hypertension.

In the present paper an investigation was made of the effect of chronic administration of high doses of guanethidine on the fluorescence histochemistry, tissue catecholamine content and functional capacity of the cardiovascular system.

METHODS

Male Sprague-Dawley rats (50 g) were treated with guanethidine (30 mg kg^{-1}, i.p. daily for 6 weeks) ($n = 37$), while control animals ($n = 24$) received corresponding injections of saline. Twenty-four hours, 1 week, 2, 4, 6 and 8 weeks after the cessation of guanethidine treatment the animals were anaesthetized with methohexital sodium (45 mg kg^{-1}, i.p.). Tracheal, left common carotid and right jugular vein cannulae were inserted and anaesthesia was maintained with pentobarbitone sodium (5 mg

kg^{-1}, i.v. as required). About 15 min after the completion of the operative procedures mean arterial blood pressure was measured with a Statham Physiological Pressure Transducer (Model P23GC). Pressor responses to occlusion of the right common carotid artery and to physostigmine (100 $\mu g\ kg^{-1}$, i.v.) were elicited and 25 min later the rats were killed by air embolism and tissues were removed for fluorescence histochemistry and catecholamine content determinations.

Ten weeks after the cessation of guanethidine treatment a second group of rats was prepared similarly and was also given a fully effective ganglion blocking dose of pentolinium tartrate (10 mg kg^{-1}, i.v.). Dose-response curves to noradrenaline were then determined by measuring the pressor response to at least five different dose levels of noradrenaline presented as single intravenous bolus doses in random order.

Fluorescence histochemistry. The catecholamines present in the ventral caudal artery, atrium and the left adrenal gland were observed by using Falck's (1962) technique for the visualization of monoamines. At least four guanethidine-treated and two control rats were used at each time interval.

The catecholamine content of the right adrenal gland and the ventricles was determined by the method of Viktora, Baukal & Wolff (1968) modified by Head & de la Lande (to be published). Adrenaline and noradrenaline were estimated separately for the adrenal gland and in the case of the ventricles total catecholamine was estimated and expressed as noradrenaline.

Drugs used were: Guanethidine sulphate (Ciba-Geigy, Aust. Ltd.); Heparin injection B.P. (Evans Medical Ltd.); (−)-noradrenaline bitartrate (K & K Laboratories); methohexital sodium (Eli-Lilly); pentobarbitone sodium (Abbott Laboratories Ltd.); pentolinium tartrate (May & Baker Ltd.); physostigmine sulphate (T & M Smith Ltd., Edinburgh).

All quantities of drugs are expressed in terms of the form of the drug named with the quantitity in the text.

Statistical procedures. Results are means (±s.e. of the mean) and tests for significance were made utilizing Student's *t*-test. Equi-effective dose ratios of noradrenaline were calculated using $\log_{10}$ doses as described by Flemming, Westfall & others (1972).

RESULTS

Fluorescence histochemistry

Twenty-four hours after the cessation of guanethidine treatment there was an absence of specific fluorescence at the adventitial-medial border of the ventral caudal artery. However, autofluorescence of the elastica was unimpaired. Two weeks after the cessation of guanethidine treatment the arterial tissues removed from half of the group of the rats showed a few spots of bright fluorescence in the adventitial-medial border. Ten weeks after cessation of guanethidine treatment ventral caudal arteries from all rats showed fluorescence in this zone, but fluorescence was still attenuated compared with controls.

The fluorescence of atria taken from control animals appeared as fine beaded threads dispersed throughout the myocardium with intense fluorescence around blood vessels. In atria taken from rats 4, 6 and 8 weeks after guanethidine treatment scattered threads were rarely seen, but a few spots of intense fluorescence did appear.

The large amounts of intense yellow and green fluorescence spread throughout the adrenal medullae of control animals was unaltered in guanethidine treated rats.

Catecholamine content

The catecholamine content (expressed as noradrenaline) of ventricles taken from control animals was 0·55 ± 0·07 μg g^{-1} (n=11). Guanethidine treatment was associated with a depletion of catecholamines such that 24 h and 1 week after the cessation of guanethidine treatment no measurable amounts of catecholamine were present. However, 2 weeks after guanethidine some catecholamine had returned (0·05 ± 0·03 μg g^{-1}, n=5) and the amount found at 8 weeks was even greater (0·12 ± 0·02 μg g^{-1}, n = 5) though this was still significantly less than controls ($P < 0·01$).

The noradrenaline and adrenaline content of the right adrenal gland (n = 9) removed from control animals was 3·4 ± 0·37 μg and 10·02 ± 0·59 μg per gland respectively. Guanethidine treatment neither altered these concentrations significantly nor modified the weight of adrenal glands.

Resting mean arterial blood pressure

Guanethidine treatment depressed the mean arterial blood pressure (Fig. 1), the depression being greater (30 mm Hg) ($P < 0·001$) 24 h after the cessation of guanethidine treatment than at 8 weeks after guanethidine (12 mm Hg) ($P < 0·05$). If the initial depression of blood pressure after guanethidine treatment was due to the complete removal of sympathetic tone to the cardiovascular system one would expect the blood pressure so attained to be similar to the blood pressure of rats given fully effective doses of a ganglion blocking agent. In fact the blood pressure of control animals given pentolinium tartrate (10 mg kg^{-1}, i.v.) was significantly less ($P < 0·001$) than rate 24 h after the cessation of guanethidine treatment (71·5 ± 2·1 mm Hg compared with 125·4 ± 3·3 mm Hg).

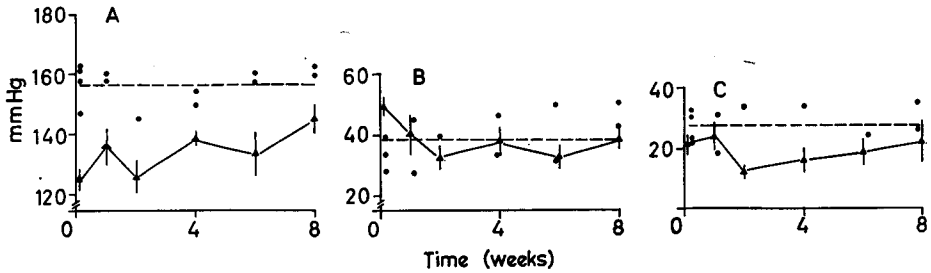

FIG. 1. Showing the effect of treatment withdrawal period on the recovery of A—the mean arterial blood pressure. B—the pressor response to physostigmine (100 μg kg^{-1}, i.v.). C—the pressor response to carotid occlusion from chronic guanethidine treatment (30 mg kg^{-1}, i.p. daily for 6 weeks) of male Sprague-Dawley rats. All measurements were made under pentobarbitone anaesthesia. The dashed line is the mean of the control values which are indicated by dots (●). Each point (▲) on the graph is the mean ±s.e. of the data from at least 4 guanethidine-treated rats. Note that 8 weeks after the cessation of guanethidine treatment the blood pressure was still significantly less ($P < 0·05$) than controls. Note also that the response to physostigmine and carotid occlusion was similar in control and guanethidine-treated rats immediately after discontinuation of guanethidine treatment when any sympathectomy might be expected to be greatest.

Pressor responses to physostigmine and carotid occlusion

Dose-response curves prepared in initial experiments revealed that the dose of physostigmine used (100 μg kg^{-1}, i.v.) produced a pressor response which amounted to 80% of the maximal pressor response elicited by this drug. The response of guanethidine-treated rats to physostigmine and to carotid occlusion (Fig. 1) showed no marked differences when compared with controls although the response to carotid

occlusion was consistently lower in guanethidine-treated animals. However, at all times after treatment a considerable response was still obtained to carotid occlusion even though at 2 and 4 weeks after the cessation of guanethidine-treatment the responses were slightly and statistically significantly depressed compared with controls ($0{\cdot}01 < P < 0{\cdot}02$ and $0{\cdot}05 < P < 0{\cdot}1$, respectively). This may simply reflect the wide range of values from this test and the relatively small number of animals used.

Pressor response to noradrenaline

As can be seen in Fig. 2, 10 weeks after the cessation of guanethidine treatment the $\log_{10}$ mean dose-response curve to noradrenaline is shifted to the left, more so at low doses than at high doses. The equieffective dose ratios for noradrenaline are shown on the graph.

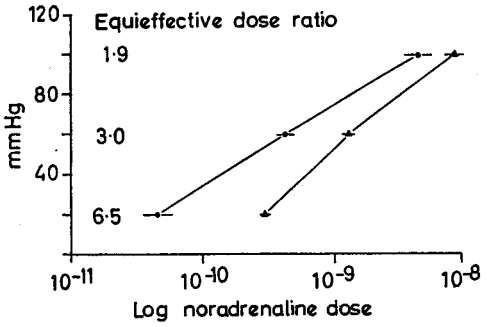

FIG. 2. Mean $\log_{10}$ dose-response curves to noradrenaline (mol kg^{-1}) *vs* increase of blood pressure in ganglion-blocked, pentobarbitone-anaesthetized male rats pretreated with guanethidine chronically (●—●) or corresponding injections of saline (▲—▲). The guanethidine treatment (30 mg kg^{-1}, i.p. daily for 6 weeks) had ceased 10 weeks before the measurement of these responses to noradrenaline. Each point was from $\log_{10}$ dose-response curves (11 control; 5 guanethidine-treated) drawn for individual rats using at least 5 dose levels of noradrenaline to construct each curve. The *P* values at the 20, 60 and 100 mm Hg response levels are $<0{\cdot}001$, $<0{\cdot}001$ and $<0{\cdot}05$ respectively.

DISCUSSION

The present study shows that guanethidine (30 mg kg^{-1}, i.p. daily for 6 weeks) causes a profound reduction in the catecholamines present in atria, ventricles and ventral caudal artery confirming the observations of Burnstock & others (1971). However, the reversibility of this effect (as evidenced by a partial return of catecholamines within 8 weeks of the cessation of guanethidine treatment) is in better agreement with the observations of Gannon, Iwayama & others (1971) who used smaller doses of guanethidine but for longer periods. Thus, fluorescence histochemistry and catecholamine determinations suggest that guanethidine does produce a sympathectomy but that it is not permanent.

Despite this action of guanethidine there is little evidence for a marked reduction in the functional capacity of the sympathetic nervous system. Mean arterial blood pressure was significantly lower in ganglion blocked animals than in animals where the sympathetic nerves had been interrupted by guanethidine treatment. Furthermore, the pressor response to physostigmine was not significantly decreased in guanethidine-treated rats and while the response to carotid occlusion was consistently less than in control animals, it was not abolished.

The pressor responses to physostigmine and carotid occlusion are thought to be mediated via the sympathetic nervous system (Medaković & Varagić, 1957; Heymans & Neil, 1958a; Lěsić & Varagić, 1961; Lalanne, Schmitt & Schmitt, 1966) and have been used as a test of the functional capacity of this nervous system (Clarke, Smookler & Barry, 1970; Finch, Haeusler & Thoenen, 1973). Carotid occlusion was thought to be particularly useful in this respect as it represented a "physiological" means of

increasing sympathetic neuronal traffic. It is possible that part of the pressor response to these treatments in guanethidine-treated animals may have arisen from the release of catecholamine from the adrenal medulla and there is evidence for such an effect with both physostigmine and carotid occlusion (Morgan, 1957; Heymans & Neil, 1958b; Lalanne & others, 1966) but other workers believe that the adrenal glands play a very minor role (Medaković & Varagić, 1957; Cass & Spriggs, 1961). The effect of any catecholamine released from the adrenal gland (where catecholamine content is unaffected by guanethidine) would, however, be potentiated by the supersensitivity present in these animals (assuming that adrenaline is affected like noradrenaline) thus enhancing any adrenal contribution.

While supersensitivity was only examined in rats 10 weeks after cessation of guanethidine, it is likely to have been as marked at 2, 4, 6 and 8 weeks, since denervation supersensitivity reaches a maximum 2–3 weeks after the procedure. The development of supersensitivity could thus explain the high arterial pressure in guanethidine-treated as compared with ganglion-blocked rats, the unchanged response to physostigmine and the minimally-changed response to carotid occlusion.

In functional terms, therefore, guanethidine appears to be ineffective in causing a sympathectomy of the cardiovascular system. This is not to say that the function of the sympathetic nerve fibre is unimpaired. It may be severely reduced but compensatory mechanisms return the function of the sympathetic effector units as a whole to something approaching normal. Functional tests must therefore always be employed to test the effectiveness of sympathectomizing agents.

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