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PHARMACOLOGY OF THE ANTIHYPERTENSIVE GUANOXAN

BY

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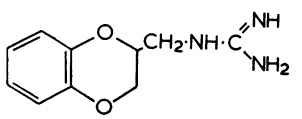
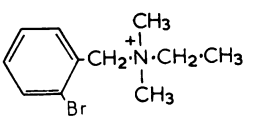
Guanoxan (2-guanidinomethyl-1,4-benzodioxan sulphate; Pfizer; Compound 3/01003, Envacar) is one of a series of compounds designed for adrenergic neurone blocking properties and synthesized in the Chemical Research Department of Pfizer (Augstein & Green, 1964).

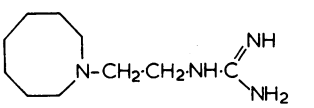
The structural formula of guanoxan is given below with the structures of bretylium and guanethidine shown for comparison.



Guanoxan



Bretylium



Guanethidine

Preliminary experiments showed that guanoxan given intravenously produced a pronounced fall in arterial pressure, blocked the carotid occlusion response and antagonized the pressor effects of afferent vagal and splanchnic nerve stimulation in anaesthetized cats and dogs. In addition a pronounced antihypertensive response was observed following the oral administration of guanoxan to conscious rats with chronic renal hypertension.

It was found, either indirectly by the method of dye dilution in dogs or directly in the Starling dog heart-lung preparation, that hypotensive doses of guanoxan were without effect on cardiac output, thus showing that the fall in blood pressure was the result of a net decrease in peripheral resistance. Since hypotensive doses of guanoxan did not alter the glomerular filtration rate and renal blood flow as indicated by continuous clearance measurements in dogs, guanoxan did not interfere with the autoregulation of renal blood flow and glomerular filtration rate.

The experiments reported below confirm that guanoxan is an adrenergic neurone blocking compound and that it produces a decrease in the noradrenaline concentration in both the central nervous system and peripheral tissues and in the catechol amine content of the adrenal glands, has an adrenolytic effect in the dog but not in the cat, and an action on central nervous system function in the anaesthetized and conscious cat. Chronic oral administration of the compound to hypertensive dogs revealed potent antihypertensive properties and inhibitory and blocking actions against indirectly acting sympathomimetic drugs. With chronic administration no tolerance to its antihypertensive or amine-depleting action could be observed in beagle dogs.

Peart & MacMahon (1964) have reported the results of a clinical trial of guanoxan in sixty-two patients with severe hypertension. Robertson's (1964) report and Genest's (personal communication, 1964) trial suggest an action on the central nervous system in addition to the antihypertensive action of the compound in man.

METHODS

Cross-perfusion of the cat isolated spleen

The method used for the cross-perfusion of the cat isolated spleen, the stimulus parameters and the techniques for collection and assay of the splenic sympathin have been described by Davey, Farmer & Reinert (1963).

Recording of ganglionic action potentials

Ganglionic action potentials were evoked by electrical stimulation of the preganglionic cervical sympathetic trunk and recorded from the superior cervical ganglion of the cat. The method employed was essentially that described by Paton & Perry (1953) using the conditions and modifications described by Reinert (1963).

Recording of the venous outflow and secretion of saliva from the cat submaxillary gland

The submaxillary gland, its duct and nerve supply were exposed according to the technique described by Liddell & Sherrington (1929). The chorda tympani was ligated and divided at the point where it left the lingual branch of the trigeminal nerve and was carefully dissected away from Wharton's duct. Wharton's duct was then cannulated with a short length of fine polyethylene tubing. Drops of saliva secreted made contact between two electrodes and operated a Thorp impulse counter. The chorda tympani and the preganglionic cervical sympathetic trunk were placed on platinum wire electrodes (cathode distal). The venous outflow from the gland was measured by the method described by Hilton & Lewis (1955). Drop formation was recorded with a Thorp impulse counter. Heparin was used as anticoagulant.

Stimulation of deep structures of the brain stem

Anaesthetized cats (acute experiments). Stereotactically placed unifocal tungsten electrodes were used to stimulate the dorsal, medial and anterior hypothalamus in cats anaesthetized with chloralose (70 mg/kg intravenously). The electrodes were prepared from 25-gauge straight tungsten rod (Tungsten Manufacturing Company) and varnished, except for the tip, with three coats of Valspar varnish. A steel needle inserted under the skin of the head served as the indifferent electrode. Muscle and skin blood flow were recorded from the right hind-limb and right fore-paw respectively using the method described by Abrahams, Hilton & Zbrożyna (1960). Mean arterial pressure was recorded by a mercury manometer from the left femoral artery. Indwelling cannulae were inserted into the lateral cerebral ventricles of cats according to the method described by Feldberg & Sherwood (1954). Spike activity in preganglionic fibres of the cervical sympathetic trunk was recorded according to the method of Iggo & Vogt (1960).

Conscious cats (recovery experiments). Anaesthesia was induced with halothane (3 to 3.5%), nitrous oxide and oxygen; the pharynx and larynx were swabbed with 1% xylocaine and a straight, cuffed endotracheal tube was inserted into the trachea; anaesthesia was maintained with halothane (0.5 to 2%), nitrous oxide and oxygen (3 : 1 v/v). Unifocal tungsten electrodes were implanted stereotactically into the dorsal, medial and anterior hypothalamus and into the nuclei of the habenula (epithalamus). The electrode mounted in a subminiature coaxial socket was secured to the skull with acrylic filling material (Sevriton; Amalgated Dental Association). The electrode carrier was then removed and the electrode was further secured with a stainless steel plate which was fixed to the skull with two stainless steel screws. A miniature coaxial socket with a thread attached served as a third screw and as the indifferent electrode. In addition the plate and screws were bonded to the skull with acrylic resin.

The electrode positions were determined histologically at the end of each experiment. The brachiocephalic artery was cannulated and the brains were perfused and fixed with 0.9% w/v saline followed by formol saline (10% w/v) containing acetic acid (3% w/v). Serial sections 20 μ thick were cut on a freezing microtome and stained with Sudan black or cresyl violet.

Measurement of relative and absolute refractory period from guinea-pig isolated atria

The method used was similar to that described by Dawes & Vane (1956). Guinea-pig isolated atria were tied to a perforated Perspex plate and placed in a Perspex chamber containing Ringer-Locke solution (g/l.: NaCl 9.0; KCl 0.42; $CaCl_2$ 0.24; $NaHCO_3$ 0.5; and glucose 1.0) at 37° C and equilibrated with oxygen.

Two rectangular wave stimulators and a Dekatron gate were adjusted so that a threshold test stimulus was introduced after every tenth conditioning stimulus. The strength of the conditioning stimulus was increased until it would just excite the preparation and the frequency was increased until the preparation followed every stimulus. The pulse duration of both the test stimulus and the conditioning stimulus was 1 msec. The interval between the test and the conditioning stimuli (initially, approximately 250 msec) was decreased in steps of 5 to 10 msec and the strength of the test stimulus was increased until a response was obtained. This arrangement enabled the determination of the effects of various concentrations of drugs on the threshold, relative conduction time and the absolute and relative refractory periods.

Conscious hypertensive dog

Male beagle dogs only were used. All surgical procedures were carried out under strict asceptic conditions. Anaesthesia was induced with 30 mg/kg of thiopentone, intravenously, followed by intubation of the trachea with a straight, cuffed endotracheal tube, and maintained throughout the operation with halothane (0.5 to 2%), nitrous oxide and oxygen (3 : 1 v/v). A carotid artery was exteriorized and the corresponding carotid sinus denervated. For complete denervation the carotid sinus nerve was sectioned and the common, internal and external carotid arteries were stripped of their sheaths for a centimetre above and below the bifurcation. All small arterial branches arising near bifurcation were severed. The vessel walls were then painted with a solution of phenol (5% w/v) and were washed with alcohol.

Systolic pressure was determined by palpation of the exteriorized carotid artery distal to an occluding sphygmomanometer cuff. The experimentally induced hypertension was either of neurogenic or nephrogenic origin.

Chronic neurogenic hypertension

The baroreceptor afferent fibres were sectioned according to the method described by Thomas (1944) The operation was performed in two separate steps.

The left carotid artery was first exteriorized, the left carotid sinus was denervated and 2 cm of the left vagosympathetic trunk were removed. The second step was performed 14 to 21 days after the first. The right carotid sinus was denervated followed by excision of 2 cm of the medial third of the right vagosympathetic trunk. It was essential to leave a sufficient number of vagal fibres intact, otherwise intractable vomiting developed with subsequent dehydration.

Since it was extremely difficult to avoid this complication, neurogenic hypertension was induced experimentally in all later experiments by the method of Derom (1958). The operation, including the exteriorization of the carotid artery, was, however, performed in one step. We have not experienced any serious postoperative complications and the survival rate has been 100%.

Chronic nephrogenic hypertension

The operation was performed in two stages. The first stage consisted of laparotomy, by midline incision, followed by mobilization of both kidneys, removal of perirenal fat and connective tissue, division of the capsular anastomotic vessels between ligatures and encapsulation of both kidneys in close-fitting moulded rubber capsules. The loose necks of the capsules did not compress the renal vessels or ureter nor did the capsules exert any constrictive effect on the kidneys. In the second stage, after laparotomy by midline incision the rubber capsules were removed. In the interval of approximately 2 weeks between the two operations the capsules provoked an intense "foreign body" reaction resulting in perinephritis and formation of a permanent perirenal fibro-collagenous capsule of 2 to 5 mm thickness.

Determination of the tissue concentrations of adrenaline and noradrenaline

Tissue concentrations of adrenaline and noradrenaline were determined by the semiautomatic method of Merrills (1962). Details of the specificity and the recoveries of catechol amines obtained with this method have been reported previously (Merrills, 1962, 1963; Davey *et al.*, 1963).

RESULTS

Adrenergic neurone block

Blood pressure in anaesthetized cats and dogs. Guanoxan (1 to 5 mg/kg) antagonized the pressor responses to splanchnic nerve stimulation, noradrenaline and tyramine and reversed that to adrenaline in dogs anaesthetized with pentobarbitone sodium (Fig. 1). This classical adrenolytic action of guanoxan was not observed in cats anaesthetized with chloralose, in which guanoxan (5 mg/kg) antagonized the effect of tyramine without altering the responses to adrenaline and noradrenaline (Fig. 2).

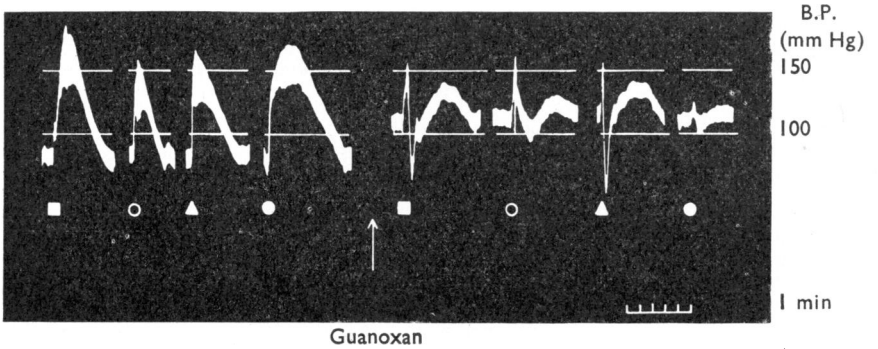


Fig. 1. Dog, 10 kg, male; anaesthetized with pentobarbitone sodium. Record of blood pressure (B.P.) responses to splanchnic nerve stimulation and to intravenous injection of noradrenaline, adrenaline and tyramine. Filled squares: supramaximal stimulation of splanchnic nerve (5 V, 0.5 msec, 16 shocks/sec for 60 sec). Empty circles: noradrenaline, 20 μg; filled triangles: adrenaline, 20 μg; filled circles: tyramine, 3 mg. At the arrow guanoxan (5 mg/kg) was given intravenously. Time marks, 1 min.

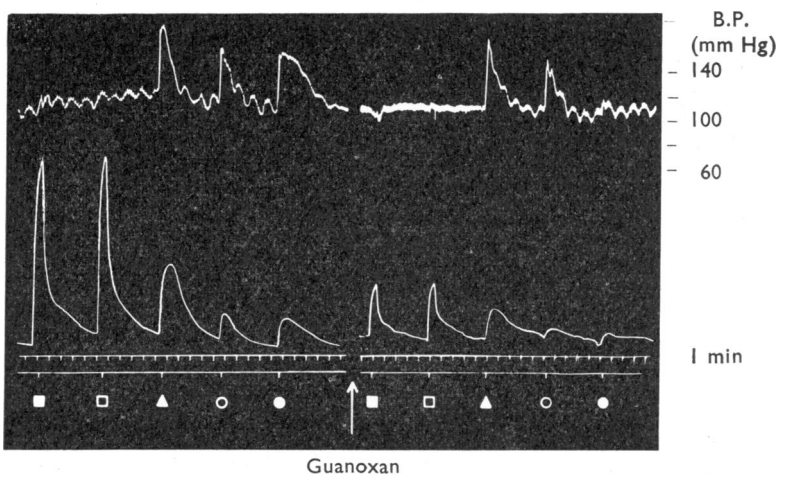


Fig. 2. Cat, 1.8 kg, female; chloralose anaesthesia. Record of arterial blood pressure (B.P., top) and contractions of nictitating membrane (bottom). Preganglionic stimulation (filled squares) and postganglionic stimulation (empty squares) of cervical sympathetic trunk (supramaximal, 0.5 msec, 16 shocks/sec for 30 sec). Filled triangles: adrenaline, 20 μg, intravenously; empty circles: noradrenaline, 20 μg, intravenously; filled circles: tyramine, 1 mg, intravenously. At the arrow guanoxan (5 mg/kg) was given intravenously. Time marks, 1 min.

Cat nictitating membrane. The subcutaneous administration of guanoxan (5 and 20 mg/kg) was followed by a marked and long-lasting relaxation of the nictitating membrane in conscious cats. Parasympathomimetic effects were absent.

Guanoxan (5 to 10 mg/kg) antagonized the contractions of the nictitating membrane elicited by stimulation of the pre- and postganglionic cervical sympathetic trunk in cats anaesthetized with chloralose (Fig. 2). No contraction of the nictitating membrane followed the intravenous injection of guanoxan, which therefore differs in this respect from guanethidine which produces a sustained contraction.

Day (1962) has shown that dexamphetamine antagonized the adrenergic neurone blocking properties of bretylium, guanethidine and xylocholine on the cat nictitating membrane. The inhibition by guanoxan of the contraction of the nictitating membrane was also antagonized by dexamphetamine sulphate (0.5 mg/kg) which suggests that guanoxan, guanethidine, bretylium and xylocholine have a similar mechanism of action on the cat nictitating membrane (Fig. 3).

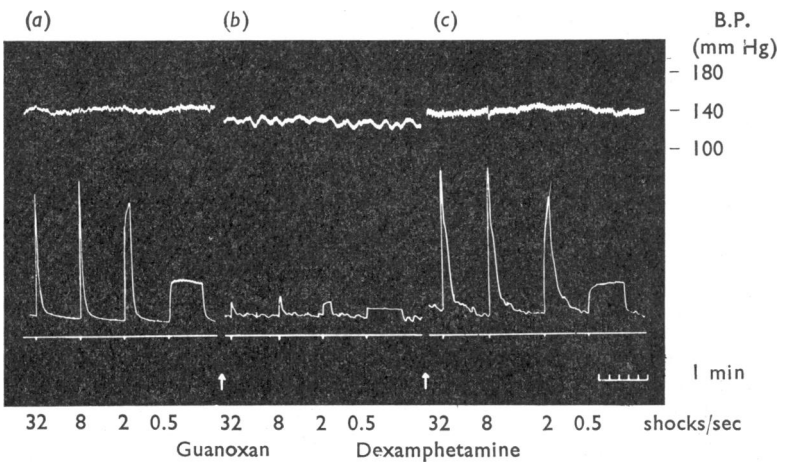


Fig. 3. Cat, 2.6 kg, male; chloralose anaesthesia. Records of arterial blood pressure (B.P., top) and contractions of nictitating membrane (bottom). Stimulation of postganglionic cervical sympathetic trunk with 100 supramaximal pulses (0.5 msec, 0.5, 2, 8 and 32 shocks/sec). (*a*) control responses; (*b*) 15 min after guanoxan (10 mg/kg, intravenously); and (*c*) 20 min after dexamphetamine (0.5 mg/kg, intravenously).

Isolated cross-perfused cat spleen. The isolated cross-perfused cat spleen, which yields in its venous outflow amounts of noradrenaline large enough for accurate assay, was used to study effects of guanoxan on the release of noradrenaline at sympathetic nerve endings. Guanoxan (2 mg) was injected directly into the arterial supply to the spleen. The venous outflow during the following 2 min was collected separately and discarded to prevent interference by the injected free guanoxan in the assay of noradrenaline. Guanoxan produced a small contraction of the spleen but no increase in the vasopressor activity of the venous blood from the spleen could be demonstrated (Fig. 4). At 5 min after the close-arterial injection of guanoxan the output of noradrenaline to stimulus frequencies of 30 and 10 shocks/sec markedly diminished and the spleen failed to contract in response to

nerve stimulation. However, the contractions of the capsular smooth muscle evoked by close-arterial injections of noradrenaline were unchanged (Fig. 4). The close-arterial injection of dexamphetamine sulphate (100 μg) restored splenic contractions to nerve stimulation and the output of noradrenaline per stimulus to near control values, the venous

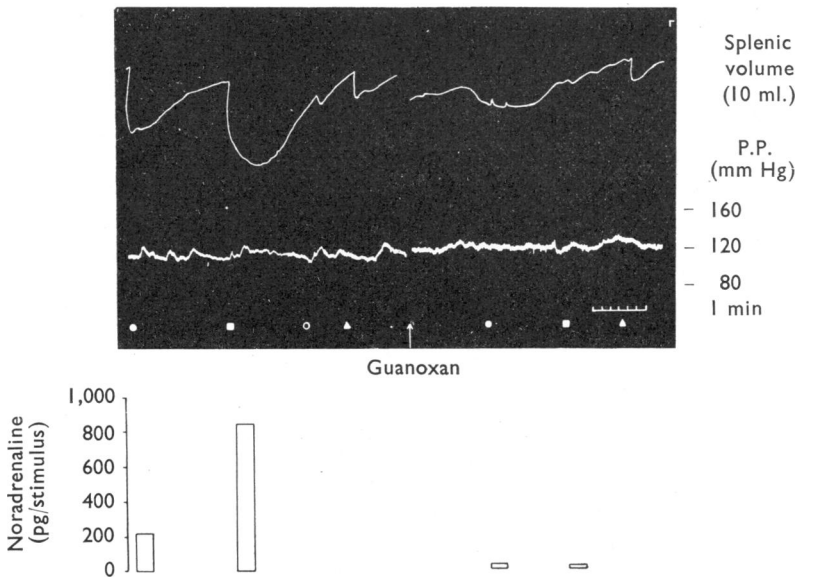


Fig. 4. Isolated perfused cat spleen; spleen cat, 2.4 kg, male; perfusion cat, 2.8 kg, male; chloralose anesthesia. Records of volume changes of the spleen, contractions downwards (top), and splenic perfusion pressure (P.P., bottom). Histogram: output of noradrenaline in venous outflow. Filled circles: splenic nerve stimulation with 200 supramaximal pulses (20 V, 0.5 msec, 10 shocks/sec). Filled squares: splenic nerve stimulation with 200 supramaximal pulses (0.5 msec, 30 shocks/sec). Empty circles: noradrenaline, 0.1 μg, close-arterially; filled triangles: noradrenaline, 0.2 μg, close-arterially. At the arrow, close-arterial injection of 2 mg of guanoxan.

outflow for the 2 min immediately following injection being discarded (Fig. 5). The antagonistic effect of dexamphetamine towards the adrenergic neurone block is therefore due to a specific restoration of the output of the sympathetic transmitter in response to nerve stimulation. In control experiments close-arterial injections of dexamphetamine sulphate (100 and 200 μg) did not alter the noradrenaline output per stimulus from the spleen at a stimulus frequency of 30 shocks/sec and in the pithed rat preparation 10 μg doses of dexamphetamine sulphate produced pressor responses equivalent to those of 5 ng of noradrenaline.

Hypothalamic stimulation and muscle blood flow. Unifocal tungsten electrodes were placed stereotactically into the hypothalamus. Electrical stimulation of the posterior hypothalamus through these electrodes evoked a rise in mean arterial pressure and a triphasic response in muscle blood flow consisting of a short-lived increase followed by a decrease and a final increase in muscle blood flow. After intravenous administration of guanoxan (5 to 10 mg/kg) the vasoconstrictor phase was abolished and hypothalamic stimulation

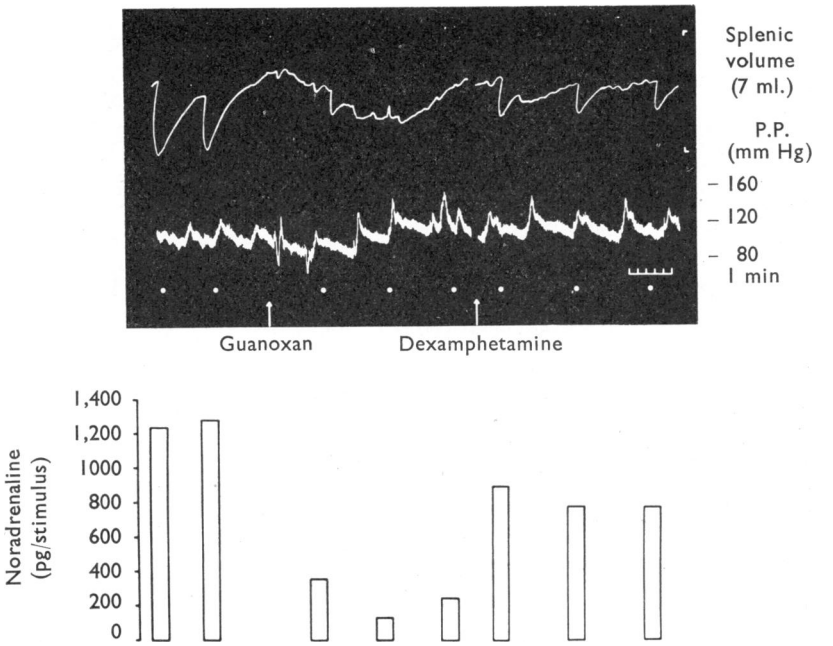


Fig. 5. Isolated perfused cat spleen; spleen cat, 2.5 kg, female; perfusion cat, 2.5 kg female; chloralose anaesthesia. Records of volume changes of the spleen, contractions downwards (top), and splenic perfusion pressure (P.P., bottom). Histogram: output of noradrenaline in venous outflow. At the white dots: splenic nerve stimulation with 200 supramaximal pulses (20 V, 0.5 msec, 30 shocks/sec). Close-arterial injection of guanoxan (2 mg) at the first arrow and dexamphetamine (100 μg) at the second arrow.

resulted in a fall in systemic blood pressure and an increase in muscle blood flow. This reversal of the effect of hypothalamic stimulation was due to the block of adrenergic vasoconstrictor fibres and the unmasking of the vasodilator effect of sympathetic cholinergic fibres (Fig. 6).

Submaxillary gland, blood flow and salivation. The cholinergic (chorda tympani) and the adrenergic (cervical sympathetic) innervations of the submaxillary gland of the cat were used as a further test for the adrenergic neurone blocking properties of guanoxan.

Vasodilatation and salivation followed electrical stimulation of the chorda and stimulation of the cervical sympathetic trunk resulted in vasoconstriction followed by vasodilatation and salivation. The vasoconstriction and salivation after stimulation of the adrenergic nerve fibres were blocked by low doses of guanoxan (1 mg/kg). Approximately ten- to twenty-times this dose of guanoxan had to be injected in order to reduce the salivation following stimulation of the chorda (Fig. 7). The doses necessary to reduce cholinergic salivation were higher than those used by Emmelin & Strömblad (1963) for guanethidine. The sympathetic or muscarinic effects on salivation reported by these authors with high doses of guanethidine or bretylium respectively were not observed with either intravenous or close-arterial injections of guanoxan.

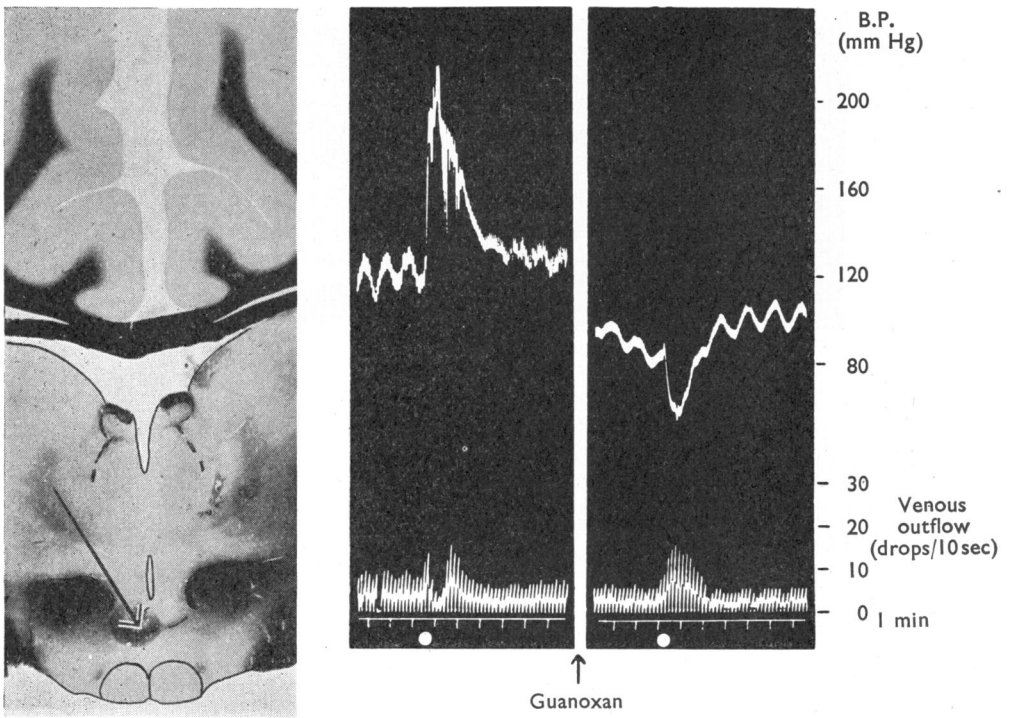


Fig. 6. Cat, 3.7 kg, male; chloralose anaesthesia. Record of mean arterial pressure (B.P., top) and venous outflow from skinned hind-limb (bottom). Filled circles: hypothalamic stimulation with supramaximal pulses (20 V, 0.5 msec, 125 shocks/sec for 20 sec). At the arrow intravenous injection of 10 mg/kg of guanoxan. On the left is a retouched photomicrograph of a coronal section of the brain with the electrode position indicated by the arrow.

Ganglionic transmission. Intravenous injection of guanoxan reduced transiently the amplitude of the evoked ganglionic action potential recorded from the superior cervical ganglion of the cat. Guanethidine (1 mg/kg) produced a greater and more persistent depression of the S1 spike than did guanoxan (5 mg/kg) (Fig. 8).

Conduction time and refractory period. The effects of guanoxan on the excitability of guinea-pig isolated atria were studied in view of the observation of Dawes (1946) that the benzdioxan F 933 had a strong quinidine-like action. Guanoxan (4×10^{-5}) did not change the relative conduction time or the relative and absolute refractory periods, whereas quinidine (1×10^{-5}) greatly increased the refractory period, reduced the excitability of the tissue and prolonged the conduction time.

Continuous infusion of guanoxan (4.50 mg/min) into anaesthetized dogs did not alter the electrocardiogram until a total dose of 80 to 90 mg/kg had been given. When this amount was infused respiratory paralysis occurred.

Sympathomimetic effects. Oral administration of single doses of guanoxan (10 mg/kg) to conscious beagle dogs caused a rise in blood pressure and tachycardia of 3 to 4 hr duration, but this effect was not repeatable. No sympathomimetic action was observed when 1 mg/kg of guanoxan was administered daily by mouth and gradually increased to 10 mg/kg.

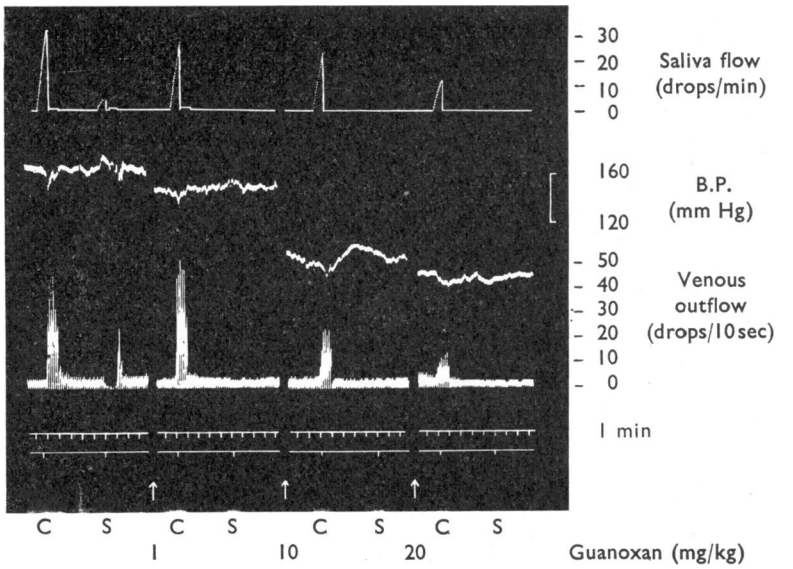


Fig. 7. Cat, 3.3 kg, male; chloralose anaesthesia. Record of salivary secretion from the submaxillary gland (top), mean arterial pressure (B.P., middle) and venous outflow (bottom). Stimulation of the chorda tympani (C) and cervical sympathetic nerve (S) with supramaximal pulses (20 V, 0.5 msec, 10 shocks/sec for 60 sec). At the arrows intravenous injection of guanoxan, 1, 10 and 20 mg/kg.

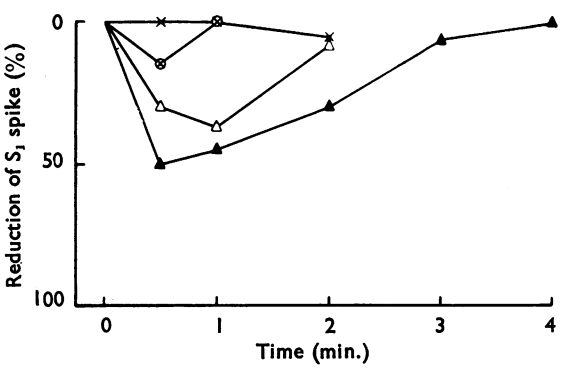


Fig. 8. Cat, 2.3 kg, male; chloralose anaesthesia. The graph represents reduction of the S_1 spike of evoked ganglionic action potentials from the superior cervical ganglion after intravenous injection of guanethidine or guanoxan at 0 min. ×——×, guanoxan (2 mg/kg); ⊗ —— ⊗, guanoxan (5 mg/kg); △ —— △, guanethidine (1 mg/kg); and ▲ —— ▲, guanethidine (2 mg/kg).

Antihypertensive action in hypertensive dogs

Acute effects. Guanoxan was administered orally to conscious beagle dogs with experimentally induced nephrogenic or neurogenic hypertension in order to study its rate of absorption, the degree and duration of its hypotensive effect, the development of tolerance and its effect against directly and indirectly acting sympathomimetic drugs. The fall in blood pressure after 10 mg/kg of guanoxan reached a maximum at 6 hr and lasted for more than 24 hr. Subsequent doses of guanoxan did not increase but prolonged the antihyperten-

sive effect (Fig. 9). The blood pressure returned to the pre-dose level 2 to 3 days after drug withdrawal. The daily administration of 10 mg/kg was optimal and an increase of the dosage did not result in a greater antihypertensive effect but produced side-effects such as diarrhoea and vomiting.

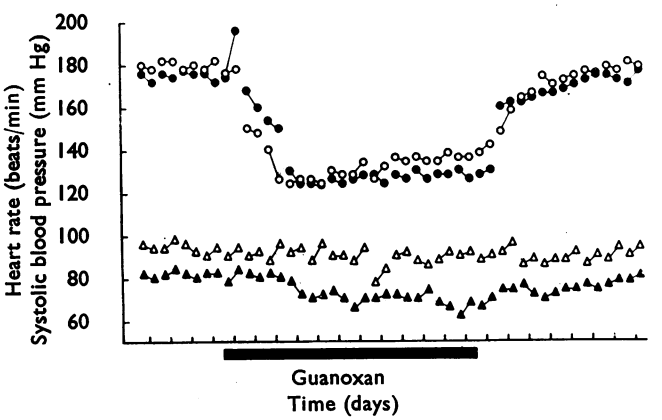


Fig. 9. Records of heart rate (triangles) and systolic blood pressure (circles) of a 17 kg male beagle with chronic neurogenic hypertension (empty symbols) and a 16 kg male beagle with chronic nephrogenic hypertension (filled symbols). The first symbol of each pair represents the morning reading and the second represents the reading obtained 6 hr later. The solid bar indicates the period of oral administration of 10 mg/kg of guanoxan. The compound was given daily approximately 1 hr after the morning blood pressure measurement.

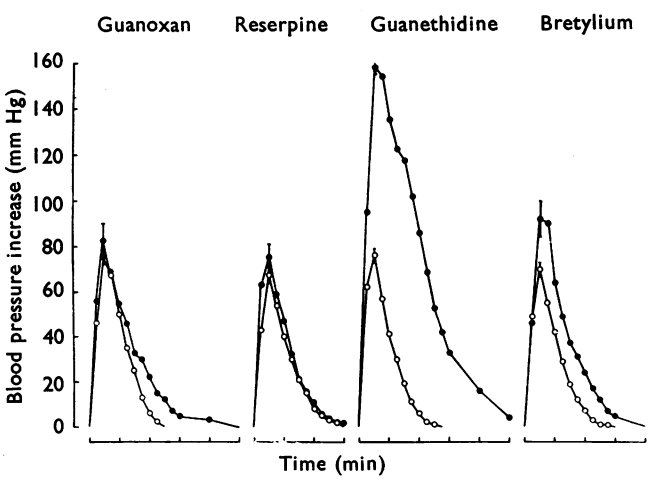


Fig. 10. Records of arterial pressor responses to single intravenous injections of 3 μg/kg of noradrenaline in groups of two nephrogenically and two neurogenically hypertensive beagles before and during oral administration of guanoxan (10 mg/kg), reserpine (250 μg/kg), guanethidine (20 mg/kg) and bretylium (40 mg/kg) given daily for 3 days. The vertical lines are the standard errors of the mean determined at the peak of each pressor response. Empty circles: mean of pressor responses before treatment; filled circles: mean of pressor responses on 3rd day of administration.

Comparison of the antagonism by guanoxan, guanethidine, bretylium and reserpine of the cardiovascular effects of dimethylphenylpiperazinium, tyramine and noradrenaline. Guanoxan (10 mg/kg), reserpine (250 μg/kg), guanethidine (20 mg/kg) and bretylium (40 mg/kg) were each administered orally daily for 3 days to two nephrogenically and two neurogenically hypertensive beagle dogs. Reproducible pressor responses to single intravenous injections of dimethylphenylpiperazinium (7 μg/kg), noradrenaline (3 μg/kg) and tyramine (200 μg/kg) were obtained before, during and after this treatment. Guanethidine and bretylium markedly potentiated the pressor effects of noradrenaline, whereas they were unchanged by guanoxan and reserpine (Fig. 10). Guanoxan, reserpine and guanethidine, but not bretylium, antagonized the pressor effect of tyramine (Fig. 11). The pressor effects of dimethylphenylpiperazinium were not affected by guanoxan, bretylium and reserpine, but guanethidine significantly potentiated the effects of dimethylphenylpiperazinium (Fig. 12).

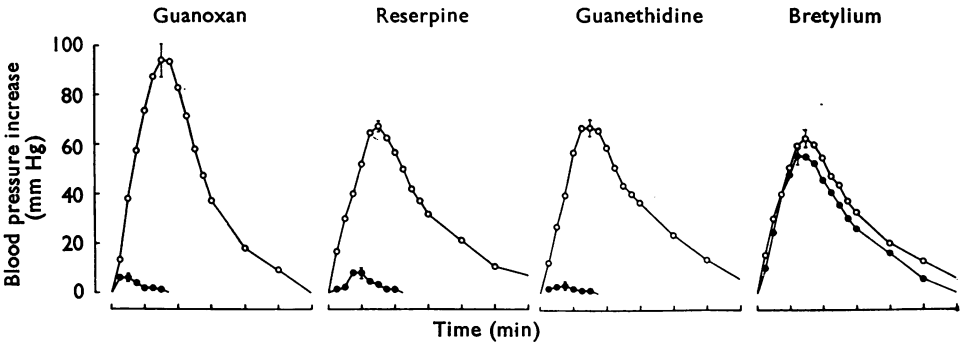


Fig. 11. Records of arterial pressor responses to single intravenous injections of 200 μg/kg of tyramine in groups of two nephrogenically and two neurogenically hypertensive beagles before and during oral administration of guanoxan (10 mg/kg), reserpine (250 μg/kg), guanethidine (20 mg/kg) and bretylium (40 mg/kg) given daily for 3 days. The vertical lines are the standard errors of the mean determined at the peak of each pressor response. Empty circles: mean of pressor responses before treatment; filled circles: mean of pressor responses on 3rd day of administration.

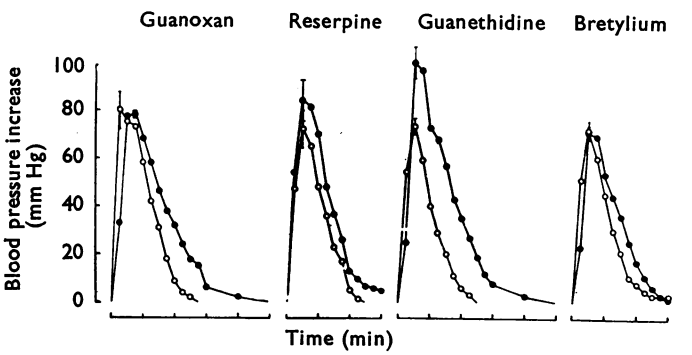


Fig. 12. Records of arterial pressor responses to single intravenous injections of 7 μg/kg of dimethylphenylpiperazinium in groups of two nephrogenically and two neurogenically hypertensive beagles before and during oral administration of guanoxan (10 mg/kg), reserpine (250 μg/kg), guanethidine (20 mg/kg) and bretylium (40 mg/kg) given daily for 3 days. The vertical lines are the standard errors of the mean determined at the peak of each pressor response. Empty circles: mean of pressor responses before treatment; filled circles: mean of pressor responses on 3rd day of administration.

The antihypertensive effects of chronically administered guanoxan in hypertensive beagle dogs. Guanoxan (10 mg/kg, orally) was administered daily to four beagle dogs with nephrogenic hypertension in order to determine whether tolerance developed toward the antihypertensive effect which would limit the clinical usefulness of the compound (Fig. 13).

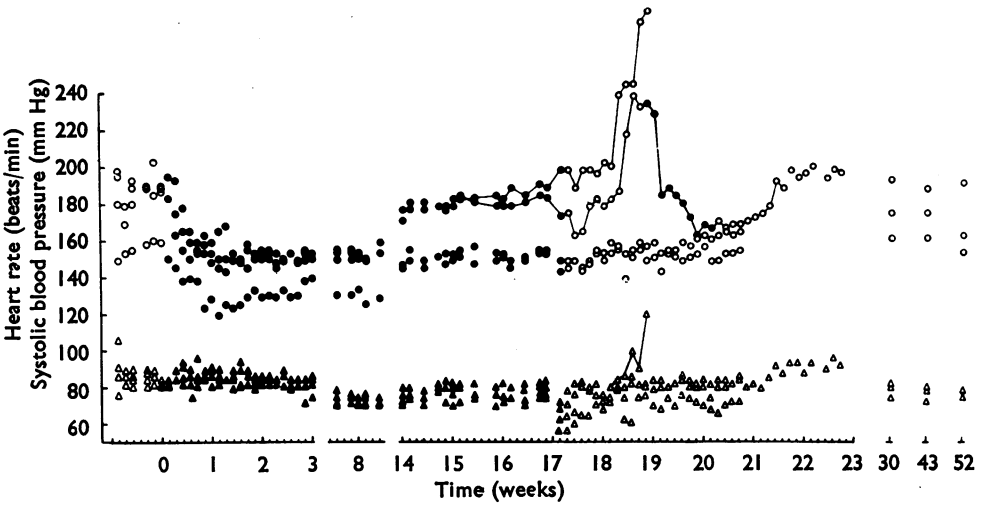


Fig. 13. Records of heart rate (triangles) and systolic pressure (circles) during oral administration of guanoxan (10 mg/kg/day) to four nephrogenically hypertensive beagle dogs. Empty symbols: glucose placebo; filled symbols: oral administration of 10 mg/kg of guanoxan.

The antihypertensive effect of guanoxan remained unchanged during the first 12 weeks of administration in all four dogs. In two dogs, however, the systolic pressures gradually returned toward the pre-dose control levels during weeks 12 to 17. At the 17th week the drug was withdrawn. The blood pressure of these two dogs rose dramatically 1 week after the cessation of the administration of guanoxan. One of these dogs died and *post mortem* revealed myocardial hypertrophy and dilatation, old and fresh kidney infarctions and chronic perinephritis (which was the result of the experimental encapsulation of the kidney). Guanoxan (10 mg/kg, orally) was immediately administered to the surviving dog and within 48 hr an effective antihypertensive response was again achieved. The drug was finally withdrawn and 2 weeks later the blood pressure started to rise towards the pre-dose control level (Fig. 13). The results demonstrate that true drug tolerance did not develop in any of these four dogs. The apparent " tolerance " was a reflection of the progression of the hypertensive disease with a corresponding gradual increase in the basic systolic pressure and was not due to a decrease of the antihypertensive activity of the compound.

Dimethylphenylpiperazinium, noradrenaline and tyramine were injected intravenously once weekly into these dogs to test if tolerance towards the amine-depleting or adrenergic neurone blocking (inhibition of amine release) properties of guanoxan did develop. The pressor responses obtained with these drugs after 4, 8 and 12 weeks' continuous administration of guanoxan are shown in Fig. 14. The response to dimethylphenylpiperazinium

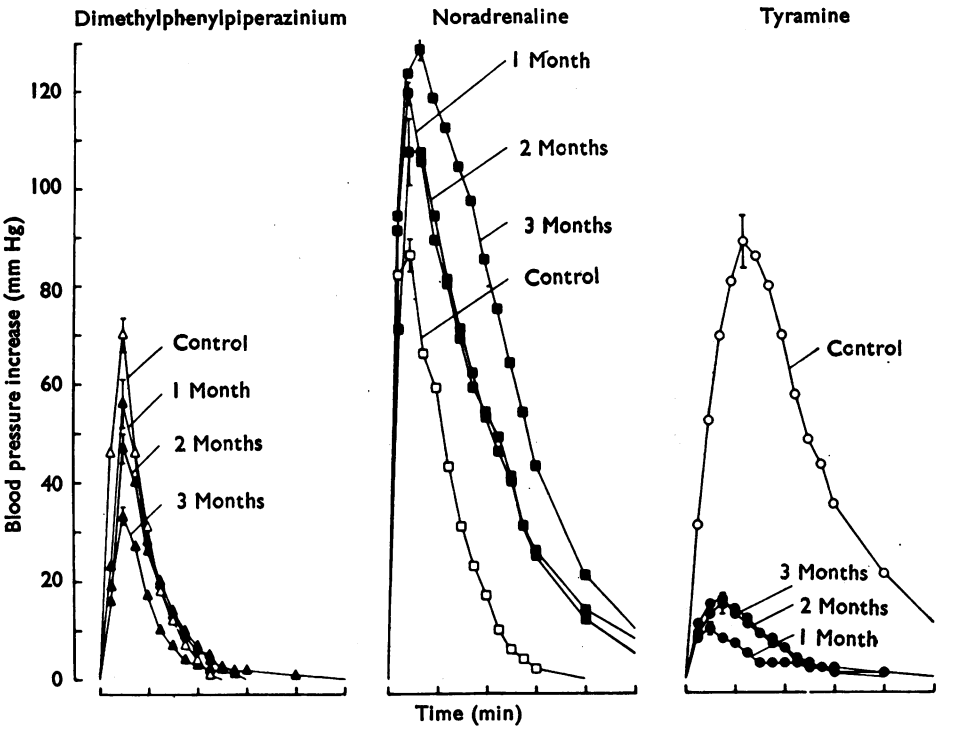


Fig. 14. Mean pressor responses to intravenous injections of dimethylphenylpiperazinium (7 μg/kg), noradrenaline (3 μg/kg) and tyramine (200 μg/kg) in four conscious nephrogenically hypertensive beagle dogs before and during prolonged oral administration of guanoxan (10 mg/kg/day). The vertical lines are the standard errors of the mean determined at the peak of each pressor response. Empty symbols: control period; filled symbols: during period of treatment.

declined progressively. After 12 weeks the effect of dimethylphenylpiperazinium was only 50% of the original despite the marked increase of the rise in blood pressure after noradrenaline. The gradual reduction of the dimethylphenylpiperazinium effects reflects to our mind the progressive decrease of the catechol amine content of the adrenal medulla.

The tyramine responses remained almost completely abolished for the whole duration of the experiment. The initial block of the tyramine effect may be based on a mechanism by which the adrenergic neurone blocking agent prevents the entry of tyramine into the granules and its exchange with the adrenergic transmitter. The block after prolonged administration of guanoxan is more likely a result of the depletion of the adrenergic transmitter in nerve endings.

The absence of tolerance to the amine-depleting effect of guanoxan was demonstrated when various tissues were analysed for their noradrenaline content at the end of 1 year's toxicity trial. The noradrenaline contents of all tissues examined were considerably reduced and there was a greater depletion in all tissues except the spleen after 12 months' than after 1 month's administration. The results are summarized in Table 1.

TABLE 1

THE EFFECTS OF DAILY ORAL ADMINISTRATION OF GUANOXAN ON TISSUE CONTENT OF NORADRENALINE IN BEAGLE DOGS

Values are means expressed as μg/g of tissue, with standard errors

Tissue	Controls (n=3)	10 mg/kg, 1 month (n=3)	10 mg/kg, 12 months) (n=5	40 mg/kg, 12 months (n=6)
Heart	0·918±0·190	0·038±0·009	0·030±0·0008	0·021±0·0003
Spleen	0·656±0·083	0·022±0·001	0·072±0·042	0·145±0·123
Adrenal glands				
Adrenaline	1378±110	410±82·9	203±46·4	106·7±6·7
Noradrenaline	335±28	183±24·7	102±20·0	98·0±12·6
Hypothalamus	1·103±0·040	0·189±0·038	0·083±0·037	0·052±0·017
Thalamus	0·335±0·005	0·107±0·009	0·069±0·014	0·054±0·009
Cortex	0·219±0·110	0·094±0·063	0·049±0·002	0·013±0·003
Mesencephalon	0·535±0·140	0·169±0·001	0·110±0·004	0·046±0·001
Myelencephalon	0·495±0·060	0·087±0·001	0·025±0·004	0·014±0·001
Amygdalae	0·085±0·005	0·036±0·005	0·025±0·008	0·006±0·0005

The effects of guanoxan on the concentration of catechol amines in the hypothalamus, myocardium and adrenal glands

Rabbits. Guanoxan (15 mg/kg) was injected intramuscularly, daily for 2 weeks. The rabbits were killed with a blow on the back of the neck. The concentrations of noradrenaline in the myocardium and hypothalamus, and of noradrenaline and adrenaline in the adrenals, were decreased by guanoxan (Fig. 15). The same dose of guanethidine administered under identical conditions did not deplete hypothalamic and adrenal catechol amines; it decreased, however, the myocardial noradrenaline concentration to a greater extent (Fig. 15).

Beagles. Twelve male beagles, divided into four groups of three dogs each, were given either guanoxan (1 mg/kg), guanoxan (5 mg/kg), guanethidine (5 mg/kg) or a glucose placebo orally daily for 4 weeks.

Guanoxan (1 mg/kg) greatly reduced the noradrenaline content of the myocardium but did not change the concentration of noradrenaline in the hypothalamus or the adrenaline and noradrenaline content of the adrenal glands (Fig. 16); with 5 mg/kg of guanoxan the decrease in the myocardial content was no greater than that produced by 1 mg/kg but a pronounced decrease of hypothalamic noradrenaline and a fall in the adrenaline content of the adrenal glands were seen with the higher dose (Fig. 16). Guanethidine (5 mg/kg) did not change the concentration of noradrenaline in the hypothalamus although the effects on the catechol amine content of the myocardium and adrenal glands were similar to those produced by 5 mg/kg of guanoxan.

Actions of guanoxan on the central nervous system

Preganglionic activity. The recording of the spontaneous potentials from nerve fibres and filaments of the preganglionic cervical sympathetic trunk in cats (Iggo & Vogt, 1960) was not suitable for the determination of a central action of guanoxan since intravenous injections of the compound produced an increase in the firing rate on account of baroreceptor reflex activity. Two different types of neurophysiological experiments were carried

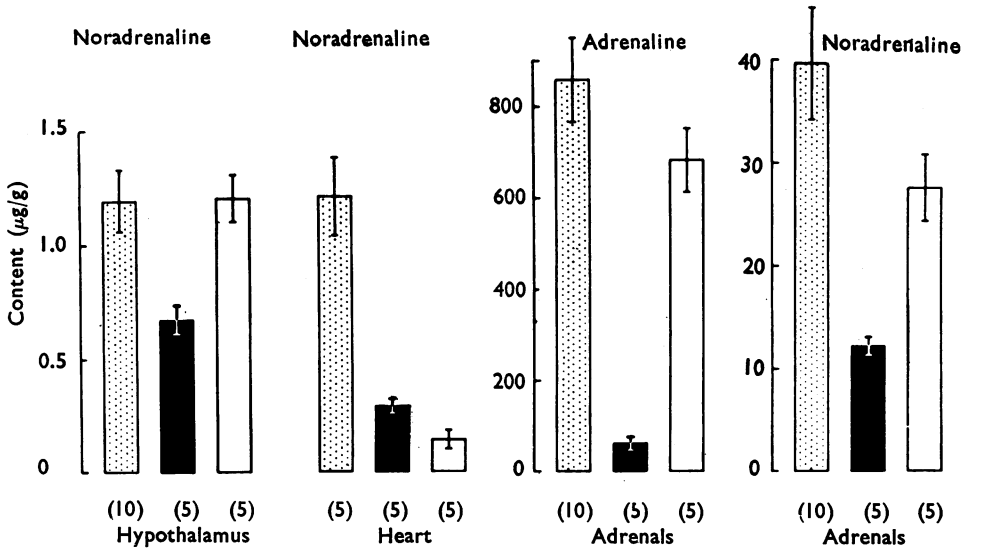


Fig. 15. Histogram of the average concentrations of adrenaline and noradrenaline in the adrenal glands and noradrenaline in hypothalami and hearts of rabbits after intramuscular injection of guanoxan (15 mg/kg) and guanethidine (15 mg/kg) daily for 14 days. The vertical lines are the standard errors of the means. The figures immediately under the columns indicate the numbers of experiments. Stippled histograms: control values; filled histograms: guanoxan (15 mg/kg/day for 14 days); empty histograms: guanethidine (15 mg/kg/day for 14 days).

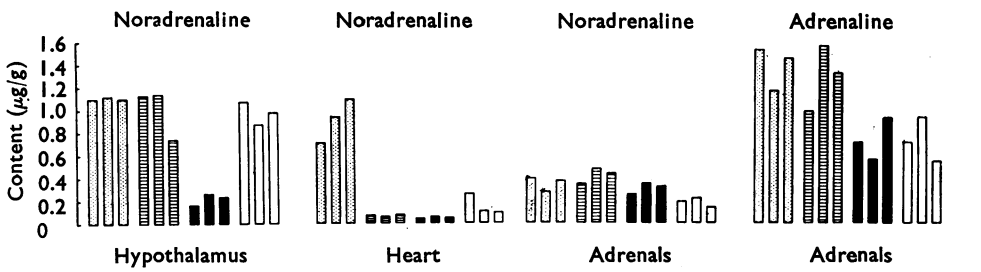


Fig. 16. Histogram of noradrenaline content of hypothalamus and heart and the noradrenaline and adrenaline content of adrenal glands of beagle dogs after oral administration of guanoxan (1 and 5 mg/kg) and guanethidine (5 mg/kg) daily for 28 days. Each column represents the value for one dog. Stippled columns: controls; hatched columns: guanoxan (1 mg/kg); filled columns: guanoxan (5 mg/kg); empty columns: guanethidine (5 mg/kg).

out in order to determine whether the compound had a central action. In the first series of experiments recruitment of vasomotor neurone activity during hypothalamic stimulation was measured in anaesthetized cats, and in the other the behavioural, somatic and autonomic responses were observed in conscious cats during stimulation of the hypothalamus or epithalamus.

Recruitment of vasomotor neurone activity. Unifocal tungsten electrodes were placed stereotactically into the hypothalamus, and an indwelling cannula was inserted into a lateral

cerebral ventricle. Action potentials were recorded from filaments of the preganglionic cervical sympathetic nerve. Electrical stimulation of the hypothalamus increased the spontaneous firing rate recorded from the preganglionic nerve fibres. Intraventricular injection of guanoxan (500 μg) reduced and finally abolished the recruitment but it had no effect on the basic spontaneous firing rate. Comparison of control values before stimulation with those in the immediate post-stimulatory period and with those at subthreshold stimulation showed that they remained unchanged during the whole experimental period (Fig. 17).

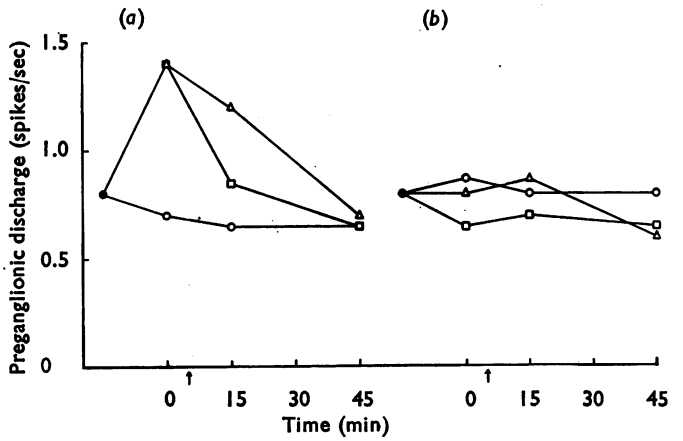


Fig. 17. Cat, 2.2 kg, female; chloralose anaesthesia. Record of spike frequency of preganglionic cervical sympathetic nerve. At the arrows injection of guanoxan (500 μg) into a lateral cerebral ventricle. Filled circles: mean of four measurements during a 45 min control period. The left graph (*a*) represents discharge frequency during hypothalamic stimulation (250 μsec, 125 shocks/sec for 20 sec). The right graph (*b*) represents discharge frequency in the immediate poststimulatory period. Empty circles: stimulation with 10 V; empty squares: stimulation with 20 V; and empty triangles: stimulation with 30 V.

Hypothalamic and epithalamic defence reaction. Electrical stimulation of the hypothalamus through permanently implanted electrodes in the conscious cat produced the characteristic somatic, autonomic and behavioural responses of the defence reaction (Hess & Brügger, 1943), namely piloerection, miosis, salivation, urination, unsheathing of claws, rage, aggression and flight. Stimulation with electrodes implanted into the epithalamus (habenular nuclei and stria medullaris) produced the same somatic and autonomic responses. The epithalamic defence reaction differed, however, from the hypothalamic defence reaction in two aspects. The autonomic, somatic and behavioural responses elicited from the epithalamus showed considerable after-discharge and the epithalamic defence reaction never culminated in flight, rage or aggression but resembled more the behavioural pattern of a frightened, cowering or withdrawing animal (Reinert, 1964).

The defence reaction was greatly reduced within 5 to 10 min of an intraventricular injection of guanoxan (300 μg). The amplitude for effective stimulation had to be increased in order to produce the behavioural response seen before the administration of the drug; furthermore the latency of the response was prolonged (reduced recruitment) and the continuance of effects after the end of stimulation of the epithalamus was decreased (reduced

after-discharge). The effect of the drug in the two types of experiments described above was very similar. In both recruitment was reduced or abolished.

Abrahams *et al.* (1960) have shown that cardiovascular changes form an integral part of the defence reaction. At the conclusion of the experiments in the conscious cats, blood pressure, skin and muscle blood flow were therefore recorded when they were anaesthetized with chloralose. Electrical stimulation of the hypothalamus or the medial nucleus of the habenula (epithalamus) through the implanted electrodes produced a rise in blood pressure, decrease in skin and increase in muscle blood flow. Intraventricular injection of guanoxan (200 to 500 μg) reduced or blocked the vasoconstrictor (adrenergic) and vasodilator (cholinergic) response without changing the mean arterial pressure.

DISCUSSION

Close-arterial injections of guanoxan abolished the contractions and reduced the output of noradrenaline in response to stimulation of the postganglionic innervation of the cat isolated cross-perfused spleen, whereas the contractions of the capsular smooth muscle produced by close-arterial injections of noradrenaline were unchanged by guanoxan. This observation confirms the adrenergic neurone blocking action of the compound. In common with xylocholine, guanethidine and bretylium (Day, 1962) the antagonistic effect of guanoxan on the contractions of the cat nictitating membrane in response to postganglionic stimulation is abolished by dexamphetamine, which suggests a common mechanism of action for xylocholine, guanethidine, bretylium and guanoxan at postganglionic adrenergic nerve endings. That this effect of dexamphetamine is due to a specific restoration of the output of noradrenaline has been shown using the cat cross-perfused spleen.

It is tempting to speculate on the mechanism of this effect of dexamphetamine. Reiner-(1960) reported that of the common sympathomimetic amines ($\pm$)-amphetamine, dext amphetamine and methylamphetamine alone produced depolarization and block in the cat superior cervical ganglion. The demonstration by Ferry (1963) that acetylcholine excites the nerve endings of sympathetic postganglionic adrenergic C fibres suggests that dexamphetamine also may act (in a fashion similar to its action on the subsynaptic membrane) at other sites in adrenergic neurones. Boura & Green (1963) have drawn an analogy between the effects of adrenergic neurone blocking agents in preventing release of catechol amines and their local anaesthetic action. Guanoxan also has a powerful and long-lasting local anaesthetic action. Perhaps because of their intrinsic affinity for adrenergic nerves, adrenergic neurone blocking agents cause a hyperpolarization of the terminal axonal membrane and thus interfere with the changes that occur in axonal membrane characteristics during the discharge of noradrenaline from granules. Repeated administration of guanoxan decreased the noradrenaline content of the heart and spleen and the catechol amine content of the adrenal glands (with a greater depletion of adrenaline than of noradrenaline) in rabbits and dogs. In addition to this depletion in peripheral tissues guanoxan produced a marked decrease in the concentration of noradrenaline in the central nervous system of rabbits and dogs.

The decrease of hypothalamic noradrenaline may represent a central action of guanoxan. It is unlikely, in our opinion, that this was the result of a reflex mechanism, as was suggested by Sanan & Vogt (1962) to account for the small but significant decrease in the hypo-

thalamic concentration of noradrenaline which they observed in rabbits treated with guanethidine. In the rabbit both guanoxan and guanethidine decreased the noradrenaline levels in peripheral tissues. The depletion of myocardial noradrenaline is initially more pronounced after guanethidine than after guanoxan, but only guanoxan decreased adrenal and hypothalamic catechol amines.

In dogs the myocardial noradrenaline is reduced to the same extent after 1 and 5 mg/kg of guanoxan and it would seem reasonable to expect a fall in the hypothalamic concentration of noradrenaline at both dose-levels were this decrease of reflex origin. Only at the 5-mg/kg level, however, did guanoxan reduce hypothalamic noradrenaline. The same argument can be applied to the results obtained with 5 mg/kg of guanethidine. This dose effectively decreased noradrenaline in peripheral tissue but did not change the hypothalamic noradrenaline concentration. The problem of a central action of guanoxan is an important point to us, and further studies are under way using ^{14}C-labelled compound, autoradiography and scanning techniques in animals and man. With these techniques it should be possible to demonstrate the presence and localization of the labelled compound in the central nervous system. In preliminary experiments ^{14}C-labelled guanoxan has been detected in the central nervous system of dogs and cats after a single intravenous dose.

Guanoxan has a persistent adrenolytic action in the dog, but in the cat this effect is very weak and short-lived. This adrenolytic action and the catechol amine-depleting effects on the adrenal glands and central nervous system which guanoxan has in addition to its adrenergic neurone blocking properties may be factors contributing to the fact that tolerance toward the anti-hypertensive effect of guanoxan had not as yet been observed. When guanoxan was administered chronically to four nephrogenically hypertensive dogs the apparent tolerance which developed in two of these dogs was due to the progression of the hypertensive disease and not to a decrease in drug action. That this is so is shown by the one dog in which the cessation of administration of guanoxan was followed by a marked rise in systolic pressure which was effectively reduced by re-administering guanoxan. The subsequent withdrawal of guanoxan from this dog was followed by an increase in systolic pressure to a level above that obtained in the pre-dose control period. Furthermore no tolerance toward the amine-depleting effect of guanoxan was found, the pressor response to tyramine remained inhibited and that produced by dimethylphenylpiperazinium declined progressively; in addition, the noradrenaline content of peripheral tissues as well as of the central nervous system was reduced to a greater extent after 12 than after 1 month's treatment.

The observation that guanoxan reduced the hypothalamic content of noradrenaline in rabbits and dogs led to an investigation for possible central actions and it was found that the recruitment of medullary vasomotor neurones after hypothalamic or epithalamic stimulation was abolished after intraventricular injection of guanoxan. The excitability of hypothalamic or epithalamic neurones was reduced but the activity of the medullary vasomotor neurone was not affected since the basic firing rate of the preganglionic vasomotor neurone remained unchanged.

It is possible that guanoxan with its known peripheral adrenergic neurone blocking effect may also inhibit the supramedullary (hypothalamic or epithalamic) " adrenergic neurone," whereas it does not inhibit the cholinergic medullary vasomotor neurone. Supramedullary regions (for example, cortex, subcortex, rhinencephalon, amygdalae, hypothalamus, epi-

thalamus, etc.) modify, modulate or override medullary reflex activity in response to exteroceptive stimuli. It is feasible that persistent, asynchronous, exteroceptive stimuli and the subsequent emotional and autonomic responses (which are more easily evoked, more pronounced and longer-lasting in some individuals) might be a factor in the development and persistence of hypertensive disease.

If the excitability of supramedullary areas is reduced then exteroceptive stimuli will become less effective in overriding the tonic reflex activity of the medullary vasomotor neurone, and a drug having such an inhibitory effect on supramedullary activity might prove to be a useful therapeutic agent in the treatment of hypertension, since it would reduce the excessive oscillations of blood pressure in response to emotion. In experimental animals guanoxan reduced the excitability of the hypothalamus and epithalamus when the drug was injected intraventricularly in very small doses, and it is possible that a similar effect could be seen after systemic administration, but no proof of such an action is yet available. The fact that oral administration of guanoxan depleted hypothalamic noradrenaline could indicate that the compound crossed the blood-brain barrier to enter the central nervous system and produced a direct central action.

SUMMARY

1. Guanoxan (2 - guanidinomethyl - 1,4 - benzodioxan sulphate) possesses adrenergic neurone blocking properties; it decreased the output of noradrenaline from the cat spleen in response to stimulation of the splenic nerve, whereas the contractions of the spleen evoked by close-arterial injections of noradrenaline remained unchanged.

2. In common with that of other adrenergic neurone blocking compounds, the adrenergic neurone blockade induced by guanoxan was reversed by dexamphetamine. This reversal resulted from the specific restoration of the output of noradrenaline in response to stimulation.

3. As a consequence of its adrenergic neurone blocking activity guanoxan abolished the effects of pre- and postganglionic sympathetic stimulation and the constrictor phase in muscle blood flow induced by hypothalamic stimulation in anaesthetized cats.

4. Guanoxan had an adrenolytic action in the dog, but virtually no such action in the cat. Oral administration of guanoxan produced a pronounced and long-lasting antihypertensive effect in conscious beagle dogs with chronic nephrogenic or neurogenic hypertension. During the chronic administration of guanoxan to hypertensive dogs, the pressor responses to tyramine were abolished and those to dimethylphenylpiperazinium progressively decreased. No tolerance developed toward the compound's antihypertensive action.

5. Repeated administration of guanoxan decreased the adrenaline and noradrenaline content of the adrenals and the noradrenaline content of the heart, spleen and hypothalamus in rabbits and dogs. This depletion was greater after 12 months' administration of guanoxan than after 1 month's administration, thus showing that tolerance did not develop toward the amine-depleting action of guanoxan.

6. Injection of guanoxan into the lateral ventricle blocked the recruitment of vasomotor activity that occurred on hypothalamic stimulation in anaesthetized cats. In conscious cats intraventricular injection of guanoxan increased the threshold for the full defence reaction elicited by hypothalamic or epithalamic stimulation.

7. The relevance of the experimental results to antihypertensive therapy is discussed.

We are indebted to Drs Davies, Farmer and Merrills and Messrs Petch and Scholfield who have been intimately associated with this work.

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