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THE SELECTIVE ACCUMULATION OF BRETYLIUM IN SYMPATHETIC GANGLIA AND THEIR POSTGANGLIONIC NERVES

BY

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A study of the distribution of [^{14}C]-labelled bretylium (*N*-*o*-bromobenzyl-*N*-ethyl-*N*,*N*-dimethyl-ammonium) in cat tissues at various times after subcutaneous injection suggests that the specificity of its blocking action on adrenergic neurones may be related to its selective accumulation in these neurones. The rate of rise and fall of concentration in sympathetic ganglia and postganglionic sympathetic nerves showed a close similarity to the time course of the blocking adrenergic neurones as manifested by relaxation of the nictitating membranes. Concentrations found were similar to those in adrenergic nerve trunks when topical application of the drug had caused a local block of conduction. Conduction in other types of nerve could be blocked by topical application, but in general they were less sensitive, heavily myelinated nerves being the most resistant.

The pharmacology of bretylium (*N*-*o*-bromobenzyl-*N*-ethyl-*N*,*N*-dimethyl ammonium) has been described by Boura and Green (1959). The drug impaired the function of adrenergic neurones in doses which did not affect the parasympathetic or central nervous systems. It has been successfully used to control hypertension in man (Boura, Green, McCoubrey, Laurence, Moulton, and Rosenheim, 1959; Smirk and Hodge, 1959; Dollery, Emslie-Smith and McMichael, 1960). This study of the distribution of the [^{14}C]-labelled drug in cats suggests that the specificity of action of bretylium may be related to its selective accumulation and retention in adrenergic neurones.

METHODS

N-^{14}*C-methyl Labelled Bretylium Iodide.*—Methyl iodide, containing a nominal 3 mC of [^{14}C] (0.77 m.mole), was condensed *in vacuo* on to 0.77 m.mole of *N*-o-bromobenzyl-*N*-ethyl-*N*-methylamine in 1.8 ml. methyl acetate. The mixture was sealed off and allowed to stand at room temperature for 24 hr. The product separated as an oil that crystallized on standing. It was filtered off, washed with methyl acetate, and used without further purification (m.p. 119 to 120° on a hot stage; yield, 219 mg.). The material, suitably diluted with inactive carrier, was combusted in oxygen and [^{14}C] was counted as carbon dioxide (Glascock, 1954). It was calculated that the undiluted material had a specific activity of 3.1 mC/m.mole≡79% of the [^{14}C]-methyl iodide used. Paper chromatography in several solvent systems followed by autoradiography revealed one radioactive spot at the position stained brown by Dragendorff's reagent. R_F values were as follows: in *s*-butanol-acetic acid-water (12:5:3), 0.75; in *n*-butanol-pyridine-water (1:1:1), 0.67; and in *n*-butanol-ethanol-10% ammonia (40:20:13), 0.58. This material was diluted with two or three parts of unlabelled bretylium iodide before use.

Tissue Sampling After Subcutaneous Administration of Bretylium Iodide.—For most experiments cats received 10 mg./kg. in saline. After a suitable time interval they were anaesthetized with ether and bled out by cutting the aorta. The blood was collected and heparin added. Tissues were dissected as rapidly as possible, taking the nerves and ganglia first, then samples of larger organs, and finally samples of the central nervous system. The dissection took about 1 hr.

Ganglia, nerves and other small samples were rapidly trimmed on filter paper, weighed on a torsion balance to 0.5 mg., and laid in porcelain boats for drying at 100°. Larger samples were weighed into tared tubes and dried over phosphorus pentoxide *in vacuo* for two days.

Topical Application to Nerve Trunks.—Labelled bretylium was applied topically to several nerve

trunks in cats under chloralose anaesthesia, in order to determine its uptake by the nerves and its effect on conduction. The latter was assessed from the response of a suitable end organ when the nerve was stimulated proximal to the portion exposed to the drug. The following nerves were examined:

(*a*) Inferior cardiac: the heart rate was followed with a Cushny myocardiograph triggering a Thorp impulse counter.

(*b*) Hypogastric: the relaxant effect of nerve stimulation on the uterus was recorded by means of a Cushny myocardiograph.

(*c*) Preganglionic cervical sympathetic: the end organ response was the contraction of the nictitating membrane, recorded isotonically with a frontal writing lever.

(*d*) Greater splanchnic: carotid blood pressure was recorded with a mercury manometer. Conduction in the nerve was considered to be impaired when the pressor effect of nerve stimulation was decreased.

(*e*) Phrenic: isometric contractions of the diaphragm were recorded.

In each of these preparations the nerve was cut and freed from surrounding tissue for a length of 2 to 3 cm., and then drawn through a glass chamber with rubber dam seals at each end. This was filled with Tyrode. The proximal end of the nerve, under warm liquid paraffin, was stimulated through platinum electrodes with supramaximal shocks at 15/sec., for periods of 30 sec. every 3 or 5 min. When the response of the end organ was constant, the fluid in the nerve bath was replaced with Tyrode containing labelled bretylium. At the times stated in Table III when the end organ response, usually diminished, was again fairly constant, the immersed segment of nerve was excised, quickly rinsed in Tyrode, blotted, weighed and dried at 100° ready for assay. In one experiment the superior cervical ganglion and the adjacent portions of its pre- and post-ganglionic nerves, freed from connective tissue but otherwise intact, were immersed in a small plastic

TABLE I

THE CONCENTRATION OF BRETYLIUM IN PERIPHERAL NERVOUS TISSUES

Values for individual cats at intervals following subcutaneous injection of 10, 6 or 50 mg./kg. of [^{14}C]-labelled bretylium iodide. Concentrations are expressed as mμmoles/g. wet tissue. 1 mμmole=0·37 μg. bretylium iodide.

Tissue	10 mg./kg.					6 mg./kg.	50 mg./kg.
	3 hr.	12 hr.	18 hr.	18 hr.	72 hr.	18 hr.	18 hr.
Adrenergic nerves							
Postganglionic cervical sympathetic	42	78	—	700	31	49	696
Hypogastric	—	174	—	—	41	61	421
Gastric	—	99	—	—	—	62	—
Inferior cardiac	22	136	—	—	19	63	196
Cholinergic nerves							
Preganglionic cervical sympathetic	28	29	—	80	8	23	114
Greater splanchnic	14	30	—	—	8	24	37
Vagus	15	19	—	40	2	—	47
Phrenic	11	94	—	40	—	—	—
Sympathetic ganglia							
Superior cervical	197	270	445	890	61	174	965
Middle cervical	—	—	—	610	—	—	—
Stellate	168	315	303	770	16	185	728
Coeliac	158	374	450	950	41	225	634
Superior mesenteric	—	59	258	700	—	—	—
Inferior ,,	290	362	—	430	49	152	440
Other ganglia							
Nodose	38	15	41	30	1	6	37
Ciliary	—	64	—	30	—	—	—
Otic	—	7	—	1	—	—	—
Dorsal root	—	16	—	10	—	—	—
Semilunar	—	<1	—	1	—	—	—

TABLE II

THE CONCENTRATION OF BRETYLIUM IN VARIOUS TISSUES

Values for individual cats at intervals after subcutaneous injection of 10 or 50 mg./kg. of [^{14}C]-labelled bretylium iodide. Concentrations are expressed as mμmoles/g. wet tissue. 1 mμmole=0·37 μg. bretylium iodide. (a) Other tissue concentrations in this cat were: parotid gland 20 mμmole, ovary 38 mμmole, thyroid 14 mμmole, cervical lymph gland 5 mμmole; (b) and (c) are the concentrations in the cortex and medulla respectively; (d) left ventricle; (e) cerebral and cerebellar cortex and spinal cord.

	10 mg./kg.							50 mg./kg.
	1 hr.	3 hr.	3 hr.	12 hr.	18 hr. (a)	18 hr.	72 hr.	18 hr.
Blood	23	15	—	13	28	17	<1	24
Liver	288	78	201	62	23	27	—	—
Kidney	113	—	—	—	9	13	—	—
Adrenal gland ..						110 (b)		
	77	53	—	20	78	164 (c)	<1	85
Heart (d)	400	131	146	44	22	32	<1	38
Spleen	205	34	102	235	142	108	25	45
Lung	—	88	—	—	—	—	—	—
Diaphragm ..	52	—	—	—	45	50	—	—
Area postrema ..	1	25	—	—	—	20	—	—
C.N.S. (e)	<1	<1	—	<1	—	<1	—	—
Hypothalamaus ..	1	3	—	—	—	—	—	—

trough. Stimulation was applied to the preganglionic nerve. After immersion in bretylium, the concentrations in the ganglion and in the immersed portions of the pre- and the postganglionic trunks were assayed separately.

Assay.—The small samples were combusted in oxygen and [^{14}C] counted as carbon dioxide (Glascock, 1954). Most of the nerve samples weighed between 7 and 10 mg. Where necessary, inactive glucose was added to bring the total weight within this range. From the measured volume of carbon dioxide produced and the specific activity, the total activity in each sample was calculated. Large samples were dried *in vacuo* over phosphorus pentoxide for several days, powdered, and plated out on polythene planchettes for counting at infinite thickness under an end window counter.

The results were calculated by reference to calibrations prepared from samples containing known amounts of activity. Most counts were taken to within 2% standard deviation, but samples of low activity had standard deviations up to ±10%.

RESULTS

Previous experiments (Boura and Green, 1959) have shown that the nictitating membranes of cats injected subcutaneously with 10 mg./kg. bretylium bromide began to relax after 3 hr., were fully relaxed at 12 and 18 hr., and regained their normal tone only after two to three days. The time course of this effect, found also in the present series of experiments with the labelled bretylium iodide, was characteristic of the slow and persistent adrenergic neurone blocking action of the drug. It was in relation to this that the distribution of bretylium at various time intervals after injection (Tables I and II) is of particular interest.

The levels of radioactivity found in sympathetic ganglia and their postganglionic trunks showed that these tissues attained far higher concentrations of the drug than did any others examined (Tables I and II). At 18 hr. after subcutaneous injection of 10 or 50 mg./kg., when the nictitating membranes were fully relaxed, these ganglia contained as much as 250 to 1,000 mμmoles/g., and their postganglionic trunks 200 to 700 mμmoles/g. The rate of accumulation of these concentrations was slow, especially by the nerve trunks. The values at 72 hr. indicate that the rate of decline was also slow. The other nerves and ganglia examined accumulated moderate concentrations, in contrast to the high concentrations found in adrenergic nerves and sympathetic ganglia.

The concentration of bretylium in blood and in some other organs is shown in Table II. One hour after injection the blood contained about 20 mμmoles/ml., and similar levels were found at 3, 12 and 18 hr., but much less at 72 hr. Differential centrifugation of the blood in siliconed tubes showed that the concentration was

approximately the same in plasma and red cells. There was no more bretylium in the platelet fraction than could be accounted for by residual plasma. Other organs, for example, liver, kidneys, spleen and heart, accumulated concentrations far exceeding that in the blood within 1 hr. Levels in most organs had greatly declined within 12 to 18 hr., but those in the adrenal gland and spleen were well maintained. No drug was found in the central nervous system except for small amounts in the hypothalamus and area postrema.

After a dose of 6 mg./kg. only partial relaxation of the nictitating membranes occurred; 18 hr. after this dose the concentrations of bretylium in the tissues were considerably less, those in sympathetic ganglia being a half to one-quarter of those in the cats given 10 mg./kg. After 50 mg./kg. of bretylium all tissue levels at 18 hr. were roughly the same as those in cats given 10 mg./kg.

The possibility was considered that bretylium might penetrate into nerve axoplasm during the course of the ionic fluxes associated with impulse propagation and that this mode of entry would account for the rather slow rate of accumulation. A cat in which the right preganglionic cervical sympathetic nerve had been cut a month beforehand was given 10 mg./kg. labelled bretylium subcutaneously. Eighteen hours later the concentration of bretylium in the superior cervical ganglion of the sectioned side (122 mμmoles/g.) was similar to that of the intact side (117 mμmoles/g.) and there was no noteworthy difference in the amounts of bretylium in the postganglionic nerves (79 and 41 mμmoles/g. respectively). In an acute experiment, where the nerve of one side was cut and the other side left intact but stimulated supramaximally for 3 hr. after intravenous injection of the drug, there was again little difference between the bretylium contents of the superior cervical ganglia with their attached postganglionic nerves (cut, 232 mμmoles/g.; stimulated, 388 mμmoles/g.).

Topical Application.—Table III summarizes experiments in which various nerves were immersed in labelled bretylium, the degree of impairment of conduction assessed from the response of end organs, and the uptake of drug measured. The inferior cardiac and the hypogastric nerves took up the drug to concentrations exceeding those in the fluid in which they were immersed, within 20 to 100 min. Stimulation of the inferior cardiac nerve containing 600 mμmoles/g. no longer affected the heart rate, but, while conduction in the hypogastric nerve was apparently impaired when it contained an overall concentration of 90 mμmoles/g., blockade was still incomplete with 1,270 mμmoles/g. The preganglionic cervical

TABLE III

CONCENTRATION OF BRETYLIUM IN NERVES AFTER TOPICAL APPLICATION

The nerve, immersed in Tyrode solution containing [^{14}C]-labelled bretylium iodide, was stimulated at 15 pulses/sec. for 30 sec. every 3 or 5 min. Concentration of bretylium calculated from radioactivity determination.

	Response Tested	Bath Concentration of Bretylium Iodide mμmoles/ml.	Immersion Time in Min.	Inhibition of End Organ Response	Concentration of Drug in Nerve, mμmoles/g.
Adrenergic nerves					
Inferior cardiac	Heart rate	500	20	Complete	600
Hypogastric	Uterus	50	100	Partial	90
,,	,,	500	75	,,	1,270
Cholinergic nerves					
Preganglionic cervical sympathetic	Nictitating membrane	500	30	None	520
,,	,,	500	30	,,	500
,,	,,	1,000	30	,,	340
,,	,,	1,000	90	Partial	310
Greater splanchnic	Rise in B.P.	500	30	,,	90
,, ,,	,, ,,	500	35	,,	50
Phrenic	Diaphragm	500	30	None	90

sympathetic nerve also readily absorbed the drug, but an impairment of conduction was apparent in only 1 of 4 preparations. It may be significant that the nerve which showed blockade, though containing no greater concentration of the drug, had been immersed 90 min. instead of 30 min. The longer period of immersion might perhaps have allowed deeper penetration into the nerve. In an experiment not shown in the Table, the drug was taken up readily not only by the preganglionic cervical sympathetic trunk but also by the superior cervical ganglion and its postganglionic nerve trunk; these structures contained 850, 420, and 540 mμmoles/g. respectively after they had been immersed together for 30 min. in bretylium at a concentration of 500 mμmoles/ml. The response of the nictitating membrane to preganglionic stimulation was abolished.

The greater splanchnic and the phrenic nerve (Table III) took up bretylium to only about a fifth of the concentration of the solution in which they were immersed, within 30 min. Some impairment of the pressor response to stimulation of the splanchnic nerve containing 50 to 90 mμmoles/g. was apparent, but supramaximal stimulation of the phrenic nerve containing 90 mμmoles/g. continued to cause maximal contractions of the diaphragm.

Similar results were obtained with topical application of unlabelled bretylium bromide. The inferior cardiac nerve was blocked by immersion in a solution of 185 mμmoles/ml. for 13 min. (one experiment), and the postganglionic cervical sympathetic nerve was blocked by 450 mμmoles/ml. in 25 min. (one experiment). No impairment of conduction was apparent in preganglionic cervical sympathetic nerves immersed for 30 min. in bretylium at 1,000 to 2,700 mμmoles/ml. (five experiments).

Discussion

The following conclusions are based on the assumption that the radioactivity found in samples was accounted for solely by unaltered drug. Bretylium has been found to suffer negligible metabolic alteration in contact with tissues (Duncombe and McCoubrey, 1960).

The most interesting feature in the distribution of bretylium after subcutaneous injection in the cat is the slow accumulation of very high concentrations of the drug by sympathetic ganglia and their postganglionic nerve trunks. Moreover, the results for various time intervals show that the levels in these tissues are temporally related to the degree of relaxation of the nictitating membrane, a manifestation of the adrenergic neurone blocking action of the drug (Boura and Green, 1959). Other ganglia and nerve tissues never attained such high concentrations of the drug, and the specificity of the blocking action of bretylium on adrenergic nerves (Boura and Green, 1959) may therefore be related to the preferential accumulation of the drug by the adrenergic neurones. The sympathetic ganglia contained higher concentrations than did their postganglionic trunks, and this may be due to their having a relatively smaller proportion of supporting tissue.

Several of the major organs in the body accumulated tissue levels far exceeding those in the blood, but the concentrations attained were considerably less than those in adrenergic neurones and, except in the spleen, reached their peak and declined before the onset of sympathetic block. In experiments in guinea-pigs and rats (McCoubrey, unpublished), the spleen did not retain high concentrations of bretylium. In general, organs known to contain high concentrations of catechol amines accumulated the drug, but there is no clear relationship between their catechol amine concentrations and their degree of retention of the drug. In particular, the concentration in the adrenal medulla was far less than that in adrenergic neurones. At the highest concentration found in the superior cervical ganglion, there were about 10 molecules of bretylium present for every molecule of noradrenaline. It is interesting that, although bretylium did not penetrate into the cerebral cortex and spinal cord, small amounts were detected in the hypothalamus and area postrema, regions known to contain noradrenaline (Vogt, 1954).

Bretylium has a very persistent local anaesthetic action and blocks conduction in the adrenergic nerve trunks supplying the rabbit intestine, uterus and ear vessels when topically applied in a concentration of 37 to 370 mμmoles/ml. (Boura and Green, 1959). The present experiments show that with topical application block occurs in the postganglionic (adrenergic) nerves of the cat when they contain an overall concentration similar to that found when blockade has been produced by a subcutaneous dose. However, the distributions within the nerve cannot be expected to be the same in the two cases. Nerves of other types could also be blocked by topically applied drug but, in general, those examined were less sensitive. The available evidence is not sufficient to show how far the degree of block is related to the thickness of nerve trunks or to the ease of access to individual fibres. The differences observed after topical application of the drug, for example the high sensitivity of postganglionic fibres and the

insensitivity of the phrenic nerve, are compatible with myelin acting as a barrier to the bretylium ion. Fibre size may also be important in relation to sensitivity, as it is with other local anaesthetics. These factors are presumably of importance not only when the drug is applied topically but also when it is given systemically.

We conclude that bretylium may act in a manner analogous to that of the local anaesthetic drugs, as was originally postulated to explain the adrenergic nerve blocking action of choline 2,6-xylyl ether bromide (Hey and Willey, 1954). Its specific action on adrenergic neurones may be related to their ability to selectively accumulate the drug, as well as to the greater sensitivity of finer and less protected fibres. It seems reasonable to assume that the whole of the adrenergic neurone is susceptible to bretylium, but that the ease of access and therefore of block will be influenced by differences in the richness of the local blood supply. The vascularity of the ganglia appears to be greater than that of nerve trunks, and this is in keeping with their more rapid attainment of a blocking concentration. The drug may also have a comparatively ready access to the nerve terminals, and after subcutaneous injection these may well suffer impairment of function first.

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