THE DISTRIBUTION AND EXCRETION BY CATS OF A NEW HYPOTENSIVE DRUG, *N*-BENZYL-*N'N"*-DIMETHYLGUANIDINE

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The distribution and excretion of ^{14}C-labelled *N*-benzyl-*N'N"*-dimethylguanidine (BW 467C60) have been studied in cats. The drug, like bretylium, had a selective affinity for adrenergic neurones. At 3 mg./kg., about half the dose was excreted unchanged in urine within 24 hr. and no metabolites were found. Excretion continued for more than 7 days.

N-Benzyl-*N'N"*-dimethylguanidine (BW 467C60) is a new hypotensive drug with pharmacological properties intermediate between those of bretylium and guanethidine (Boura and Green, 1962). Bretylium localises in the adrenergic neurones of cats (Boura, Copp, Duncombe, Green and McCoubrey, 1960), a property shared by two analogues of the drug (Boura, Duncombe and McCoubrey, 1961). We wished to know whether BW 467C60 also shared this property. For the study of distribution the drug was labelled with ^{14}C at the benzyl carbon atom. The same material was used to measure the urinary excretion by cats and to search for metabolites.

Materials

Unlabelled drugs were supplied by Dr. E. Walton who also kindly supplied details of the preparation of BW 467C60 from benzylamine.

[*Carboxy-^{14}C]benzoic acid.* Phenyl magnesium bromide absorbed [^{14}C]carbon dioxide to give benzoic acid in 95·5 per cent yield with a specific activity of 5·1 mc/mmole.

[*Carbonyl-^{14}C]benzamide.* The above benzoic acid (60 mg.; 2·5 mc) was diluted with inactive benzoic acid (40 mg.) and refluxed for 3 hr. under dry nitrogen with thionyl chloride (0·3 ml.) and dry benzene (0·3 ml.). Additional dry benzene (1 ml.) was added and the mixture was evaporated at about 50 mm. pressure. The residue was transferred in dry benzene (2 ml.) to a tube connected to a vacuum manifold. It was cooled to about −40° and the air pumped out. The tube was disconnected from the manifold by stopcock and the manifold was then filled with dry ammonia. The gas was admitted to the tube in small quantities with shaking and cooling as required, until no more was absorbed. The semi-solid mass was treated with water (2 ml.) and extracted with benzene (3 × 5 ml.). Inactive benzamide (23 mg.) was dissolved in the aqueous residue by warming and the extraction repeated. The mixed benzene extracts were evaporated to give a white residue of benzamide (121 mg.; 99 per cent yield).

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[α-^{14}C]*Benzylamine.* The above benzamide was refluxed with lithium aluminium hydride (135 mg.) in dry ether (5 ml.) under dry nitrogen for 5 hr. Excess hydride was destroyed by moist ether followed by 5N sodium hydroxide (5 ml.). Benzylamine was extracted by ether (4 × 3 ml.), the extract dried over sodium sulphate, and distilled. Yield 82·5 mg.; 77 per cent.

N-[α-^{14}C]*Benzyl*-N'N"-*dimethylguanidine hydriodide.* The above benzylamine was refluxed with methyl isocyanate (56 mg.) in ether (5 ml.) for 10 min. and the solvent evaporated. The residue was refluxed with methyl iodide (1 ml.) in ethanol (4 ml.) for 20 min. and the ethanol and excess iodide evaporated. The residue was refluxed with ethanolic methylamine ((33 per cent; 7 ml.) with addition of more methylamine (1 ml.) every 30 min. during 3·5 hr. Ethanol was distilled and the residue diluted with ether. After standing for 2 hr. the product was centrifuged down (204 mg.). It was crystallised from ethanol-ether as yellowish prisms, m.p. 190–194° (181 mg.). Radioassay indicated 2·36 mc/mmole, equivalent to 56 per cent overall yield from benzoic acid based on ^{14}C used.

Autoradiography of chromatograms developed in s-butanol : acetic acid : water (11 : 5 : 3) revealed a single compact spot R_F 0·78, the same as authentic BW 467C60 visualised by Dragendorff's reagent.

Methods

Doses of BW 467C60 were given to cats by stomach tube under light ether anaesthesia.

Tissue samples were taken at 16 hr. after the dose as described for bretylium (Boura, Copp, Duncombe, Green and McCoubrey, 1960). The radioactivity of small samples (<30 mg. dry weight) was measured by Schöniger combustion and liquid scintillation counting (Kelly, Peets, Gordon and Buyske, 1961) using ethanolamine as CO_2-trapping reagent (Jeffay and Alvarez, 1961). Larger samples were dried over P_2O_5 under moderate vacuum for several days. They were powdered and plated in polythene planchettes for counting at infinite thickness.

Urines were diluted with an equal volume of ethanol, cooled to 0° and filtered. Aliquots (40 μl.) were plated on lens paper in planchettes for counting under a mica end window counter. Standards were prepared for each sample by adding labelled BW 467C60 to an aliquot sufficient roughly to double the counting rate.

Results

Tissue Distribution

Table I shows the concentrations of BW 467C60, calculated from the radioactivity present, in the larger organs of two cats at 16 hr. after a subcutaneous dose (3 mg./kg.) of the drug. The bile of one cat (1·3 ml.) contained radioactivity equivalent to 3·1 μg. of the drug.

Table II shows the concentrations of BW 467C60 found in the nerves and ganglia of these cats. The nictitating membranes of both were almost fully relaxed when they were killed.

Urinary Excretion

The urine of one cat used to study distribution contained radioactivity equivalent to 5·9 mg. of the 9 mg. dose given. Autoradiographs of chromatograms developed in s-butanol : acetic acid : water (11 : 5 : 3) or propanol : 10 per cent ammonia (6 : 4) revealed one spot only at R_F 0·78 and 0·84 respectively, the same positions occupied by BW 467C60. In butanol : pyridine : water (1 : 1 : 1) two spots were revealed, one well marked at R_F 0·82, corresponding to unchanged drug, and one weak spot at R_F 0·70. This last result could not be repeated subsequently.

TABLE I

CONCENTRATIONS OF *N*-BENZYL-*N'N''*-DIMETHYLGUANIDINE (BW 467C60) IN CAT TISSUES AT 16 HR. AFTER A SUBCUTANEOUS DOSE

Dose: 3 mg./kg. of *N'*-[α-^{14}C]benzyl labelled drug
Concentrations are expressed as mμmoles/g. wet tissue
Values from two cats

Adrenal	17.5, 53.2	Kidney	1·3, 1·4
Lung	6·7, 16·4	Cerebral cortex	0·3, 0·0
Spleen	14·5, 14·0	Cerebellar cortex	0·4, 0·0
Left ventricle	6·9, 11·8	Hypothalamus	Trace, 0·0
Liver	4·5, 6·8	Spinal cord	2·0, 0·0
Diaphragm	5·4, 5·4	Blood	1·3, 1·7
Thyroid	2·8, 2·2	Area postrema	1·9, –
Pancreas	2·3, 2·2	Pituitary	52·0 –
Parotid	– 6·6	Lymph gland	– 0·8
Renal fat	– Trace	Cerebrospinal fluid	– 0·0

In further experiments the urine was collected at intervals of 24 hr. after subcutaneous or oral doses. The samples were assayed for radioactivity and radioautographs prepared if sufficient activity was present. Table III shows the results. In all instances save one, the urines gave single spots that travelled slightly more slowly than marker spots prepared from aqueous solutions of the drug. Since the lower R_F value corresponded to that of *N*-benzyl-*N'*-methylguanidine (BW 783C60), a search was made for this possible metabolite of the drug. The 24, 48 and 72 hr. specimens from orally dosed cats were pooled, evaporated to about 50 ml. and extracted with ethanol (100 ml.). The process was repeated using acetone. The extract was evaporated and passed down a column of Zeo-Karb 226 (H) (25 × 2 cm.) and bases were eluted by 2N hydrochloric acid. The radioactive fractions were evaporated to dryness *in vacuo* and the residue was chromatographed on Whatman 3MM paper in s-butanol : acetic acid : water (11 : 5 : 3). The single radioactive zone was eluted from the paper and upon evaporation gave an oily residue (92 mg.) with radioactivity corresponding to 5·0 mg. of BW 467C60. This resisted further efforts at purification.

The picrates of BW 467C60 and BW 783C60 were prepared by diluting ethanolic solutions of picric acid plus the drugs with an equal volume of water. BW 467C60 picrate had m.p. 146–148° (Found: N, 20·4. $C_{16}H_{18}N_6O_7$ requires N, 20·7 per cent). BW 783C60 picrate had m.p. 128–129° (Found: N, 21·2. $C_{15}H_{16}N_6O_7$ requires N, 21·4 per cent).

The above oily residue (70 mg.) was dissolved in acetone (3 ml.) and two portions (1 ml.) were pipetted onto the above picrates (0·25 g.)

respectively. Specific activities of the picrates, estimated by plating 40 μl. of the above solutions onto lens paper, were 1275 $\pm$ 59 (BW 467C60) and 1284 $\pm$ 14 counts/min./mg. (BW 783C60). After three crystallisations from 50 per cent ethanol the specific activities were respectively 1147 $\pm$ 32 and 72 $\pm$ 7 counts/min./mg. The bulk of the radioactivity in the urine extract can be accounted for as BW 467C60 though the

TABLE II

CONCENTRATIONS OF *N*-BENZYL-*N'N''*-DIMETHYLGUANIDINE (BW 467C60) IN NERVES AND GANGLIA OF CATS AT 16 HR. AFTER A SUBCUTANEOUS DOSE

Dose: 3 mg./kg. of the *N*-[α-^{14}C]benzyl labelled drug
Concentrations are expressed as mμmoles/g. wet tissue. Values from two cats

Sympathetic ganglia		*Adrenergic nerves*	
Superior cervical	43·0, 28·4	Postganglionic superior cervical	33·0, 21·6
Stellate	31·0, 59·3	Hypogastric	46·0, 33·8
Coeliac	31·0, 11·7	Inferior cardiac	40·0, 67·2
Inferior mesenteric	14·5, 43·5	Splenic	30·0, 75·4
		Gastric	– 49·6
Other ganglia		Colonic	– 16·4
Nodose	– 2·3		
Ciliary	7·1, 8·6	*Other nerves*	
Dorsal root	1·4, 3·7	Vagus	5·2, 2·6
Semilunar	3·8, –	Preganglionic superior cervical	2·1, 5·7
		Sciatic	2·0, 3·1
		Greater splanchnic	9·3, 10·8

possibility exists that about 10 per cent may be the monodemethylated product. Comparison of chromatograms of normal cat urine containing authentic BW 467C60 with those of aqueous drug showed that the urinary constituents slightly retarded the drug (R_F 0·81 and 0·77 respectively). Addition of the oily extract to the same specimen of normal urine gave a spot at R_F 0·77.

Radioassay of respired carbon dioxide from a rat kept in a metabolism apparatus after receiving the labelled drug (1 mg.) intraperitoneally showed that 0·08 μg. was completely oxidised during 24 hr. and 0·81 μg. in the subsequent 24 hr. (total 0·89 μg. = 0·09 per cent of the dose).

TABLE III

EXCRETION OF *N*-BENZYL-*N'N''*-DIMETHYLGUANIDINE (BW 467C60) IN CAT URINE
Figures are mg. drug as hydriodide calculated on radioactivity present

Dose	Time after dose							Total excreted	Per cent of dose
	24 hr.	48 hr.	72 hr.	96 hr.	120 hr.	144 hr.	168 hr.		
9·3 mg. s.c.	5·25	1·18	0·284	0·128	0·095	0·024	—	6·961	75
8·4 mg., s.c.	4·15	0·80	0·95	—	—	—	—	5·90	70
9 mg., oral after feeding	1·39	0·90	0·12	0·203	0·040	0·050	0·010	2·713	30
9 mg., oral after fasting	3·38	0·87	0·42	0·108	0·046	0·028	0·017	4·869	54

DISCUSSION

The distribution of BW 467C60 in cats at a time when there is a maximum effect on the nictitating membranes resembles that of bretylium (Boura, Copp, Duncombe, Green and McCoubrey, 1960) and its analogues (Boura, Duncombe and McCoubrey, 1961). The amounts in adrenergic nerves and ganglia were considerably higher than in other nerves and

ganglia and, on the whole, were higher than in other tissues. Assuming that the drug was present solely in the aqueous phase of an adrenergic ganglion the concentration there varied from about 1·3 to 6·6 $\times$ 10^{-5}M. The comparable figures for bretylium and BW 172C58 are 2·87 to 9·9 $\times$ 10^{-4}M and 3·8 to 6·0 $\times$ 10^{-6}M respectively. It seems that anti-adrenergic activity of these three drugs in the cat is associated with their specific affinity for adrenergic nerve tissue though, as concluded previously (Boura, Duncombe and McCoubrey, 1961), there is no simple quantitative relationship. It is possible that an adequate concentration of the drug is needed in the aqueous phase of the adrenergic tissue to maintain a loose chemical combination of the drugs with some unknown tissue component by mass action. Other hypothetical explanations are possible but information is needed on the detailed disposition within a ganglion to reach any firm conclusion. It is interesting that those tissues with high noradrenaline content, liver, spleen, and heart, had relatively high concentrations of these drugs, though there was little affinity for the adrenal.

BW 467C60 appears to escape metabolic modification and its urinary excretion showed no peculiar characteristics.

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References

Boura, A. L. A., Copp, F. C., Duncombe, W. G., Green, A. F. and McCoubrey, A. (1960). *Brit. J. Pharmacol.*, **15**, 265–270.
Boura, A. L. A., Duncombe, W. G. and McCoubrey, A. (1961). *Ibid.*, **17**, 92–100.
Boura, A. L. A. and Green, A. F. (1962). *Ibid.*, **19**, 13–41.
Jeffay, H. and Alvarez, J. (1961). *Analyt. Chem.*, **33**, 612–615.
Kelly, R. G., Peets, E. A., Gordon, S. and Buyske, D. A. (1961). *Anal. Biochem.*, **2**, 267–273.