J. Pharm. Pharmac., 1971, **23**, 327–331 *Received November* 18, 1970

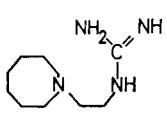
Antihypertensive and noradrenaline-depleting effects of guanethidine metabolites

L. MAÎTRE, M. STAEHELIN AND H. BRUNNER

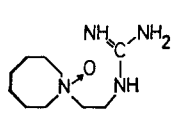
Biological Research Laboratories of the Pharmaceutical Division of CIBA-GEIGY Limited, Basle, Switzerland

The antihypertensive and myocardial noradrenaline-depleting activities of the three identified guanethidine metabolites were compared with those of guanethidine itself. Metabolite A (guanethidine-*N*-oxide) and Metabolite B [2-(6-carboxylamino)ethylguanidine] both showed approximately 1/30th of the antihypertensive effect of guanethidine in rats. Metabolite A, but not Metabolite B, caused a depletion of cardiac noradrenaline stores. The intensity of this effect was between 1/10th and 1/30th that of guanethidine. Metabolite C [(6-carboxyhexyl)-2-iminoimidazolidine] was inactive on both parameters. [^{3}H]Noradrenaline uptake into isolated bovine nerve granules was not impaired by either guanethidine or its metabolites. It is concluded that the antihypertensive and myocardial noradrenaline depleting effects of guanethidine are produced by the unchanged drug rather than by one of the identified metabolites.

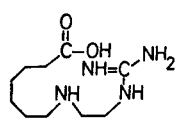
Guanethidine is widely used as an antihypertensive drug as well as a pharmacological research tool in the study of peripheral adrenergic mechanisms. Investigations have been made in the rat and in man on its excretion, distribution or metabolism or both (Dollery, Emslie-Smith & Milne, 1960; Bisson & Muscholl, 1962; Schanker & Morrison, 1965; Brodie, Chang & Costa, 1965; Furst, 1968; Rahn & Dayton, 1969). Only very recently, however, three metabolites have been identified from animal studies (McMartin, 1969) and their presence was demonstrated in the urine of hypertensive patients under guanethidine therapy (McMartin, Rondel & others, 1970). The structures of these metabolites are given below:



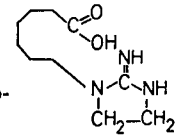
Guanethidine



Guanethidine *N*-oxide
= Metabolite A



2-(6-Carboxyhexylamino-ethylguanidine
= Metabolite B



(6-Carboxyhexyl)-2-imminoimidazolidine
= Metabolite C

The present investigation was undertaken to determine the influence of these metabolites on arterial blood pressure of renal hypertensive rats and on noradrenaline storage mechanisms.

MATERIALS AND METHODS

Antihypertensive effects

Renal hypertension was produced in male rats, of 120–140 g, according to Goldblatt, by clamping the left renal arterial with silver clips (lumen 0·2 mm). Systolic blood pressure was measured under light ether anaesthesia by the plethysmographic method

of Wilson & Byrom (1939). The experiments started at least 5 weeks after operation, with groups of rats whose mean blood pressure ranged between 190 and 225 mm Hg. Guanethidine and its metabolites were injected subcutaneously once daily for 4 days. Blood pressure was measured 2 and 24 h after each of the first three injections and 2 h after the fourth injection.

Estimation of endogenous noradrenaline

The renal hypertensive rats were killed by decapitation 2·5–3 h after the last injection of guanethidine or a metabolite. In another series of experiments, normotensive male albino rats, 180–220 g, were treated with guanethidine or its *N*-oxide (metabolite A) and killed 24 h later. The hearts were homogenized in 10% trichloroacetic acid using a Polytron PT 20 OD homogenizer and centrifuged. Noradrenaline of the supernatant was adsorbed onto alumina at pH 8·6, eluted from it with 0·25 N HCl and estimated fluorometrically according to the trihydroxyindole procedure of Euler & Lishajko (1959), but using 10 N NaOH rather than 5 N NaOH for the formation of noradrenolutine (Anton & Sayre, 1962). The recovery of noradrenaline added to heart homogenates averaged 88·2% (n = 4). No correction for incomplete recovery has been made.

Estimation of [^{3}H]*noradrenaline uptake into isolated bovine splenic nerve granules*

Bovine splenic nerve granules were isolated by differential centrifugation with some modifications (Maître, Staehelin & Bein, 1970) of the methods described by Euler (1958) and by Schümann (1958). Briefly, the procedure consists of incubating suspensions of the granule fractions at 37° for 20 min in a medium containing 0·3 M sucrose, 0·009 M sodium phosphate buffer pH 6·8, 0·003 M ATP, 0·003 M $MgCl_2$ and 0·1 mM (±)-[^{3}H]noradrenaline (0·12 μCi/μg). After incubation, the granules were collected by passage of the suspensions through a HAWP 025 Millipore filter, dried and counted in a Packard Tri-Carb liquid scintillation spectrometer after addition of 10 ml of a 0·6% butyl-PBD (Scintillator CIBA) solution in toluene. Granule suspensions kept in an ice bath during incubation time were used as controls.

The (±)-noradrenaline-[1-^{3}H]* (6·5 Ci/mmol) was obtained from New England Nuclear, Boston, Mass., USA. It was diluted with cold (±)-noradrenaline (Fluka, Buchs, Switzerland). Guanethidine was used as sulphate. The metabolites of guanethidine were kindly put at our disposal by Dr. D. F. Elliott, CIBA Horsham.

Statistical significance of observed differences was analysed with the Student's *t*- test.

RESULTS

Antihypertensive effects

Guanethidine lowered blood pressure in a dose-dependent manner. For each dose the maximal effect was reached after the third injection. The blood pressure levels measured 24 h after the second and the third injection were still markedly lower than the initial values. This reflects the long duration of action of guanethidine (Table 1). The limited availability of the metabolites did not allow assay of more than one or two doses of each metabolite. Taking into consideration the dose-dependent antihypertensive effects of guanethidine, the results presented in Table 1

* 2-Amino-1-(3,4-dihydroxyphenyl)-[1-^{3}H]ethanol.

Table 1. *Antihypertensive effects of guanethidine and of three guanethidine metabolites in renal hypertensive rats.*

Substance and dose mg/kg, s.c.		Initial b.p. (mm Hg)	Change in blood pressure (mm Hg) 2 h after the 1st application	24 h after the 1st application	2 h after the 2nd application	24 h after the 2nd application	2 h after the 3rd application	24 h after the 3rd application	2 h after the 4th application
NaCl 0·9%	(9)	212±3	− 3±3	− 6±4	−11±3	+ 1±2	−11±2	− 5±2	− 2±5
Guanethidine									
0·3	(4)	218±9				−28±6	−34±5	−19±4	−34±5
1	(4)	218±10				−33±1	−63±4	−38±2	−65±6
3	(12)	188±2	−34±6	−38±4	−64±4	−48±4	−70±4	−52±5	−69±5
6	(7)	198±6	−21±9	−35±6	−68±10	−56±10	−79±7	−74±15	−73±10
Metabolite A									
30	(4)	203±5	−31±4	− 8±7	−39±1	−28±10	−53±4	−35±7	−56±2
Metabolite B									
10	(4)	200±6	−25±5	− 3±1	−29±5	−21±10	−39±9	−13±13	−33±2
Metabolite C									
10	(4)	218±5	−16±4	− 8±4	−23±5	−14±10	−20±5	− 6±5	−11±6
30	(6)	226±6	− 8±2	− 2±3	−10±5	+ 3±2	−13±4	+ 3±2	−16±4

Substances were injected subcutaneously once daily for 4 days.
Blood pressure was measured plethysmographically under ether anaesthesia.
Figures represent mean values ± s.e.
( ) = number of rats.

indicate that metabolites A and B displayed antihypertensive activity, the intensity of which corresponded to approximately 1/30th of that of guanethidine. Metabolite C was inactive. The results further suggest that the duration of effect of metabolites A and B is of the same order of magnitude as that of guanethidine.

Effects on endogenous noradrenaline content

Renal hypertensive rats. The effects of a four days' treatment of renal hypertensive rats with guanethidine or its metabolites on noradrenaline content in heart and brain are shown in Table 2. Guanethidine, at a daily subcutaneous dose of 3 mg/kg, reduced myocardial noradrenaline content by about 80%. Among the three metabolites, only metabolite A (30 mg/kg each day) produced a depletion of endogenous noradrenaline in the heart. The intensity of depletion was of the same order of magnitude as that seen after guanethidine at a dosage 1/10th of that of metabolite A. Metabolites B and C were inactive. The noradrenaline content of brain was not altered by any substance.

Table 2. *Effects of guanethidine and metabolites on the content of endogenous noradrenaline in heart and brain of renal hypertensive rats.*

Treatment (mg/kg daily)	n	Noradrenaline μg/g heart	brain
NaCl 0·9%	7	0·76 ± 0·050	0·408 ± 0·022
Guanethidine 3	6	0·14 ± 0·015***	0·366 ± 0·031
Metabolite A 30	4	0·16 ± 0·018***	0·470 ± 0·024
Metabolite B 10	4	0·72 ± 0·032	0·396 ± 0·045
Metabolite C 10	4	0·77 ± 0·090	0·437 ± 0·032
Metabolite C 30	6	0·80 ± 0·041	0·424 ± 0·018

The substances were administered subcutaneously once daily for 4 days. The organs were removed 2·5–3 h after the last injection.
n = number of extracts.
*** = $P < 0{\cdot}001$.
The rats whose blood pressure values are shown in Table 1 were used for these determinations.

Normotensive rats

For a better estimation of the relative depleting effects of guanethidine and metabolite A, normal rats were given a single injection of each and the cardiac noradrenaline content was determined 24 h later. The results show that metabolite A was at least 30 times less potent than guanethidine (Table 3).

Table 3. *Effect of a single dose of guanethidine or metabolite A on the noradrenaline content of the rat heart.*

Substance	mg/kg s.c.	n	Noradrenaline μg/g
NaCl 0·9 %		9	0·88 ± 0·047
Guanethidine	2	3	0·45 ± 0·068***
	6	3	0·25 ± 0·010***
	10	7	0·17 ± 0·021***
Metabolite A	20	3	0·81 ± 0·033N.S.
	60	3	0·64 ± 0·090*

The hearts were removed 24 h after treatment.
n = number of extracts.
N.S. = not significant ($P < 0{\cdot}05$).
* = $0{\cdot}01 < P < 0{\cdot}05$.
*** = $P < 0{\cdot}001$.

Effects on isolated nerve granules

Guanethidine inhibits the uptake of noradrenaline into sympathetic neurons *in vivo*. But it does not inhibit the uptake into isolated nerve granules *in vitro* (Maître & Staehelin, 1970). The effect of guanethidine *in vivo* might possibly be due to the action of a metabolite. It was therefore of primary interest to determine whether the known metabolites might impair the uptake mechanism *in vitro*. All three metabolites as well as guanethidine itself were unable to diminish significantly the uptake of [^{3}H]noradrenaline into isolated nerve granules up to concentrations as high as 10^{-3} M.

DISCUSSION

Metabolite A as well as guanethidine itself lowered arterial blood pressure on renal hypertensive rats and depleted myocardial noradrenaline stores with a potency which corresponded to less than 1/30th of that of guanethidine. Metabolite B produced a moderate antihypertensive effect. At a daily subcutaneous dosage of 10 mg/kg, its action was similar to that of 0·3 mg/kg daily of guanethidine subcutaneously (Table 1). The myocardial noradrenaline content was not diminished after repeated treatments with the mentioned doses of metabolite B (Table 2) whereas a four days' oral treatment with guanethidine (10 mg/kg daily) caused a 65% depletion of myocardial noradrenaline stores. It seems therefore that the influence of guanethidine and metabolite A on blood pressure and on catecholamine stores are not shared by metabolite B. Metabolite C did not show any activity on either parameter.

An inhibiting effect of guanethidine on noradrenaline uptake in adrenergic nerve granules has been proposed by Lindmar & Muscholl (1964). Although this view was substantiated by experiments *in vitro* made on ventricle slices (Shore & Giachetti, 1966) little is known about the exact effect of guanethidine itself on isolated granules. Studies on adrenomedullary granules showed that only very high concentrations of

guanethidine are able to inhibit noradrenaline uptake (Carlsson, Hillarp & Waldeck, 1963; Lundborg & Stitzel, 1968) and experiments on nerve granules failed to demonstrate an influence of the drug on the noradrenaline release rate even at concentrations of 10^{-3} M (Euler & Lishajko, 1962). In our experiments, guanethidine also failed to diminish the uptake of [^{3}H]noradrenaline into isolated splenic nerve granules up to a concentration of 10^{-3} M (Maître & Staehelin, 1970). Since *in vivo* effects of guanethidine might be due to its metabolites it was essential to determine the direct influence of the available metabolites on the noradrenaline uptake at the granular levels. But, as now shown, the three metabolites were as inactive as guanethidine itself. It is therefore unlikely that they are involved in such a mechanism.

Recent experiments on subcellular distribution of [^{3}H]guanethidine and metabolites in the rat heart showed that these metabolites were mainly present outside the granules. After treatment of the rats with guanethidine doses which completely normalized high blood pressure, the granule fraction contained only guanethidine (Maître & Staehelin, 1970). Therefore, it can be concluded that the antihypertensive effects as well as the noradrenaline depletion are due to unchanged guanethidine and not to any of its identified metabolites.

Acknowledgements

We wish to thank Dr. D. F. Elliott, CIBA, Horsham, for the metabolites of guanethidine, and Mrs. G. Schlicht, Miss E. Wyss, Mr. B. Fehr, and Mr. P. DeHerdt for their excellent technical assistance.

REFERENCES

ANTON, A. H. & SAYRE, D. F. (1962). *J. Pharmac. exp. Ther.*, **138**, 360–375.
BISSON, G. M. & MUSCHOLL, E. (1962). *Arch. exp. Path. Pharmac.*, **244**, 185–194.
BRODIE, B. B., CHANG, C. C. & COSTA, E. (1965). *Br. J. Pharmac. Chemother.*, **25**, 171–178.
CARLSSON, A., HILLARP, N.-Å. & WALDECK, B. (1963). *Acta physiol. scand.*, **59**, *Suppl.* 215.
DOLLERY, C. T., EMSLIE-SMITH, D. & MILNE, M. D. (1960). *Lancet*, **2**, 381–387.
EULER VON, U. S. (1958). *Acta physiol. scand.*, **43**, 155–166.
EULER VON, U. S. & LISHAJKO, F. (1959). *Ibid.*, **45**, 122–132.
EULER VON, U. S. & LISHAJKO, F. (1962), *Biochem. Pharmac.*, **9**, 77–84.
FURST, C. J. (1968). *Br. J. Pharmac.*, **32**, 57–64.
LINDMAR, R. & MUSCHOLL, E. (1964). *Arch. exp. Path. Pharmak.*, **247**, 469–492.
LUNDBORG, P. & STITZEL, R. E. (1968). *Acta physiol. scand.*, **72**, 100–107.
MAÎTRE, L. & STAEHELIN, M. (1970). *Biochem. Pharmac.*, in the press.
MAÎTRE, L., STAEHELIN, M. & BEIN, H. J. (1970). *Ibid.*, **19**, 2875–2892.
MCMARTIN, C. (1969). *Ibid.*, **18**, 238–243.
MCMARTIN, C., RONDEL, R. K., VINTER, J., ALLAN, B. R., HUMBERSTONE, P. M., LEISHMAN, A. W. D., SANDLER, G. & THIRKETTLE, J. L. (1970). *Clin. Pharmac. Ther.*, **11**, 423–431.
RAHN, K. H. & DAYTON, P. G. (1969). *Biochem. Pharmac.*, **18**, 1809–1816.
SCHANKER, L. S. & MORRISON, A. S. (1965). *Int. J. Neuropharmac.*, **4**, 27–39.
SCHÜMANN, H. J. (1958). *Arch. exp. Path. Pharmak*, **233**, 296–300.
SHORE, P. A. & GIACHETTI, A. (1966). *Biochem. Pharmac.*, **15**, 899–903.
WILSON, C. & BYROM, F. B. (1939). *Lancet*, **1**, 136–139.