Biochemical Pharmacology, Vol. 29, pp. 113–115.

0006-2952/80/0101-0113 $02.00/0

Inhibition of *S*-adenosyl methionine decarboxylase by guanethidine*

(*Received* 31 *May* 1979; *accepted* 9 *July* 1979)

Guanethidine is a clinically used guanidinium adrenergic neuron-blocking agent. When administered chronically to rats at high doses, guanethidine is selectively incorporated into sympathetic neurons and destroys the sympathetic nervous system, thus producing a permanent chemical sympathectomy [1–3]. As part of an effort to determine the mechanism of this cytotoxicity, we examined the possibility that guanethidine might inhibit polyamine biosynthesis. The polyamines (putrescine, spermidine and spermine) are broadly linked to cell proliferation and growth, although their exact mode(s) of action and role in these processes are not clear [4, 5]. In this paper, we report that guanethidine is a potent inhibitor of *S*-adenosyl-L-methionine decarboxylase (SAM DC), one of the rate-limiting steps in polyamine biosynthesis [4, 5]. The characteristics of this inhibition are compared to those of the most widely studied inhibitor of SAM DC, methylglyoxal bis(guanylhydrazone) (MGBG), and the effects of guanethidine on polyamine levels in sympathetic ganglia and adrenal medulla are determined.

The effects of guanethidine sulfate (kindly supplied by Ciba Pharmaceutical Co., Summit, NJ) on the rate-limiting enzymes in polyamine biosynthesis, *l*-ornithine decarboxylase (EC 4.1.1.17) and SAM DC (EC 4.1.50), were determined in extracts of rat prostate. Rat ventral prostates [3.1 g] from approximately fifteen Sprague–Dawley rats were homogenized in 15 ml of cold (4°) buffer (25 mM potassium phosphate, 0.3 mM EDTA and 1 mM dithiothreitol, pH 7.0), using a Brinkman polytron. The homogenate was centrifuged at 10,000 *g* for 20 min. The resulting supernatant fraction was centrifuged at 100,000 *g* for 75 min, the pellet discarded, and the supernatant fraction dialyzed at 4° for 20 hr against 2 litres of buffer. The dialysate was centrifuged at 500 *g* for 5 min and aliquots of the dialyzed supernatant fraction were frozen at −10° for subsequent assay. Partially purified rat thymus diamine oxidase was prepared essentially as described by Hölttä *et al.* [6].

Enzyme assays. L-Ornithine decarboxylase and SAM DC were assayed by a CO_2-trapping method described by Beaven *et al.* [7]. SAM DC was measured in the presence of 100 mM potassium phosphate (pH 7.0), 1 mM dithiothreitol and at varying concentrations of *S*-adenosyl-L-[carboxyl-^{14}C]methionine (Amersham/Searle, Arlington Heights, IL) and putrescine (Sigma Chemical Co., St. Louis, MO), as described in the legends to the figures. The decarboxylase reactions were carried out at room temperature under conditions linear with time (60 min) and enzyme concentration. Diamine oxidase activity was determined by the method of Tryding and Willert [8], using putrescine as substrate.

Polyamine (putrescine, spermidine and spermine) levels were determined in pairs of superior cervical ganglia (SCG) or single adrenal medullae which were homogenized in cold water, and an aliquot was removed for protein analysis by the method of Lowry *et al.* [9]. The homogenates were acidified to 0.3 N $HClO_4$ and centrifuged. To 50 μl of the extract was added 25 μl of saturated Na_2CO_3 and 100 μl of dansyl chloride (5 mg/ml in acetone); this mixture was allowed to stand in the dark at room temperature overnight. Excess dansyl chloride was removed by adding 25 μl proline (250 mg/ml) and incubating the extract for 3 hr at room temperature. The dansylated polyamines were extracted with 250 μl toluene. Aliquots (180 μl) were taken, evaporated to dryness in a rotary vacuum centrifuge, and redissolved in 20 μl toluene; 10 μl were spotted on silica gel plates. Separation of the dansylated polyamines was achieved by chromatography in ethyl acetate–cyclohexane (2:1). After drying, the fluorescent spots were scraped from the plates and eluted into 1.4 ml methanol–25% NH_4OH (99:1). Fluorescence was determined on an Aminco–Bowman spectrophotofluorometer at wavelengths

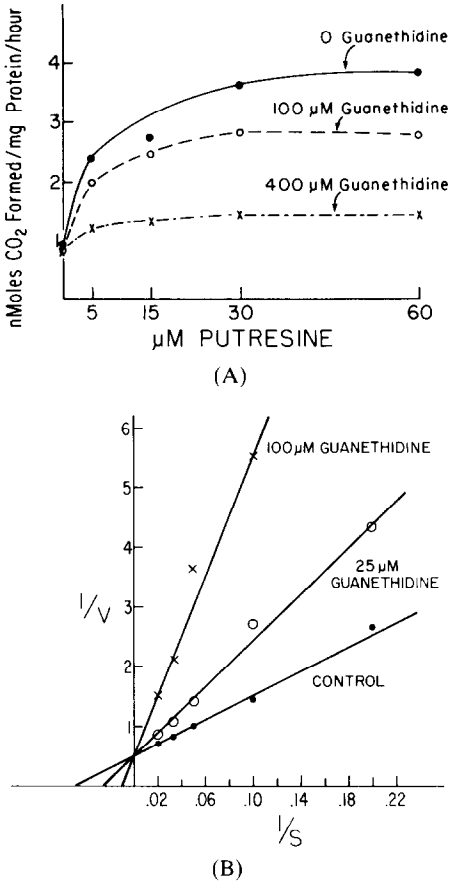

Fig. 1. Panel A: Effects of guanethidine on the activity of SAM DC from rat prostate at varying concentrations of putrescine. The assay was carried out as described in the text in the presence of 55 μM [^{14}C]SAM. Panel B: Lineweaver–Burk plot of the effect of guanethidine on putrescine-stimulated SAM DC activity (nmoles CO_2 formed/mg of protein/hr) from rat prostate at varying concentrations of SAM (μM). The assay was carried out as described in the text in the presence or absence of 1 mM putrescine. The K_i of guanethidine calculated from this data is 25 μM. The K_m of SAM is approximately 20 μM.

* This work was supported by USPHS Grant HL20604, by the National Foundation March of Dimes, by funds provided by Hoffmann-La Roche, and by NIH Training Grant HL07275.

of 365 nm and 520 nm (excitation and emission, uncorrected), and the polyamines were quantitated by comparison with standards.

The formation of [^{14}C]CO_2 from [^{14}C]SAM (carboxyl labeled) in tissue extracts is greatly increased in the presence of putrescine. The [^{14}C]CO_2 formation in the absence of putrescine is not due to the direct decarboxylation of SAM (SAM DC activity), but rather appears to be the result from the ultimate end products of the demethylation–transulfuration pathways [10]. The effect of guanethidine on the formation of [^{14}C]CO_2 from [^{14}C]SAM by rat prostate extracts is shown in Fig. 1A. In this experiment, carried out in the presence of 55 μM SAM, putrescine increased the formation of [^{14}C]CO_2 from [^{14}C]SAM approximately 5-fold, with maximal CO_2 formation achieved in the presence of 30 μM putrescine. Guanethidine had little or no effect on the formation of [^{14}C]CO_2 in the absence of putrescine, but markedly depressed the putrescine-stimulated activity (SAM DC activity) in a concentration-dependent manner. Increasing the concentration of putrescine up to 1 mM did not prevent the guanethidine inhibition, clearly showing that guanethidine is not competitive with putrescine.

The data in Fig. 1B demonstrate that the inhibition of SAM DC by guanethidine is competitive with SAM. The K_m of SAM is approximately 20 μM and the K_i of guanethidine in this experiment was 25 μM. The results of several experiments using guanethidine concentrations up to 400 μM always resulted in competitive inhibition with a K_i of guanethidine between 25 and 60 μM. The inhibition of SAM DC by guanethidine is reversible by dialysis and is not reversed by the addition of pyridoxal phosphate (data not shown). Guanethidine produced a similar inhibition of SAM DC in extracts from SCG or adrenal medulla (data not shown). In contrast to the potent inhibition of SAM DC, Guanethidine was found *not* to inhibit the other rate-limiting enzyme in polyamine biosynthesis, ornithine decarboxylase, or to inhibit the methylating enzymes, phenethanolamine-*N*-methyltransferase, catechol-*O*-methyltransferase or hydroxyindole-*O*-methyltransferase, at concentrations up to 1.6 mM (data not shown).

The ability of guanethidine to inhibit putrescine-stimulated SAM DC activity competitively with SAM is similar to that of MGBG, although the K_m of MGBG is lower (1 μM, [6]). MGBG has been widely used as an experimental tool to probe the role of polyamines in biological processes. Therefore, we compared guanethidine with respect to some of the known properties of MGBG: (a) the ability *in vivo* to stabilize SAM DC [6] and thereby increase tissue levels of the enzyme; and (b) the ability of MGBG to inhibit diamine oxidase [6].

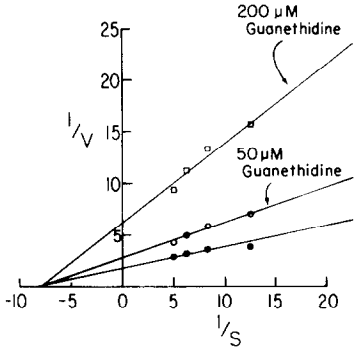

Fig. 2. Lineweaver–Burk plot of the inhibition of rat thymus diamine oxidase by guanethidine. Substrate concentrations are expressed as mM [^{14}C]putrescine and V as nmoles of product formed/mg of protein/hr. The K_i of guanethidine was 90 μM and the K_m of putrescine was 130 μM.

Five-day-old rats were treated for 3 days with a single daily injection of guanethidine sulfate (50 mg/kg, s.c.) or MGBG (Sigma; 25 mg/kg/day, s.c.). These times were chosen because maximal concentrations of guanethidine are achieved by 2 days [11] and maximal increases in SAM DC activity in other tissues by MGBG are also produced within 2 days [6]. SAM DC activity in SCG (expressed as nmoles CO_2 formed/pair/hr in the presence of 25 μM SAM and 1 mM putrescine) was not different in guanethidine-treated animals (0.53 ± 0.04, N = 3) compared to controls (0.45 ± 0.05, N = 3), whereas a 6-fold increase in activity was observed in ganglia of MGBG-treated animals (2.81 ± 0.14, N = 3). Therefore, guanethidine does not produce a stabilization of SAM DC such as that produced by MGBG.

Guanethidine does share, however, the property of inhibition of diamine oxidase (Fig. 2). The inhibition of rat thymus enzyme, as with MGBG (K_i = 1 μM; [6]), is noncompetitive and the calculated K_i of guanethidine is about 90 μM.

Despite the fact that MGBG is a potent inhibitor of SAM DC, it has been found to produce only a modest decrease in spermidine content of tissues *in vivo* at tolerated

Table 1. Effects of guanethidine or MGBG treatment on the levels of polyamine in the superior cervical ganglia of neonatal rats*

Treatment	No. of injections	Polyamine (nmoles/mg protein) Putrescine	Spermidine	Spermine
Guanethidine				
Control (4)	2	1.52 ± 0.16	8.69 ± 0.93	11.21 ± 0.96
Guanethidine (5)	2	1.37 ± 0.20	9.37 ± 1.19	10.00 ± 1.32
Control (5)	3	1.36 ± 0.15	9.08 ± 0.81	10.55 ± 0.88
Guanethidine (5)	3	2.25 ± 0.21†	9.97 ± 0.95	11.29 ± 0.83
Control (5)	4	1.32 ± 0.06	8.66 ± 0.94	10.12 ± 0.78
Guanethidine (5)	4	1.80 ± 0.19‡	11.99 ± 1.91	11.87 ± 1.70
MGBG				
Control (10)	3	2.40 ± 0.17	8.16 ± 0.43	7.97 ± 0.53
MGBG (10)	3	4.79 ± 0.44†	6.37 ± 0.61‡	5.19 ± 0.60†

* In separate experiments, rats were treated with guanethidine (50 mg/kg, s.c.) or MGBG (25 mg/kg, s.c.) daily starting the day after birth. The treated animals and the saline-treated controls were killed 24 hr after the last injection, and polyamine levels in SCG were determined as described in the text. Each value represents the mean ± S.E.M. of the number of pairs of ganglia shown in parentheses.

† $P < 0.01$ (two-tailed Student's t-test).

‡ $P < 0.05$.

doses. Since guanethidine treatment results in high concentrations (about 0.5 mM) in ganglia of both adult and neonatal rats [11, 12] and in adrenal medulla [13], it was expected that guanethidine would produce a significant decrease in polyamine levels in these tissues. The data in Table 1 show that treatment of neonatal animals with guanethidine for up to 4 days produces only a modest rise in putrescine and no decrease in spermidine and spermine levels in SCG. In contrast, MGBG (Table 1) produced a doubling of putrescine levels and a modest, but statistically significant, decrease in spermidine (22 per cent) and spermine (35 per cent) concentrations in ganglia. Similarly, treatment of adult rats for up to 10 days with guanethidine (40 mg/kg/day, i.p.) caused no decrease in spermidine or spermine levels in adrenal medulla (data not shown). These results suggest that inhibition of polyamine synthesis is not involved in the cytotoxic effects of guanethidine. This conclusion is also supported by our failure to overcome the cytotoxic effects of guanethidine with maximally tolerated doses of spermidine, spermine, combinations of the two, or by exogenous administration of SAM at doses which produce marked increases in SAM levels in the ganglia (data not shown). In addition, MGBG administration, at doses which decrease spermidine and spermine levels (Table 1), is not cytotoxic *in vivo* to sympathetic neurons.

It is unclear why guanethidine fails to lower polyamine levels in ganglia and adrenals. It is present in these structures at total concentrations which are far in excess of the K_i for *in vitro* inhibition. Inhibition of diamine oxidase is probably of no consequence since ganglia do not contain detectable amounts of the enzyme (data not shown). This may suggest that much of the guanethidine is bound (perhaps in storage vesicles) and that the free concentration in the cytoplasm is much smaller. Alternatively, it may indicate that polyamines turn over very slowly in sympathetic neurons, which accumulate guanethidine selectively and which are almost all post-mitotic by birth in the rat [14]. The decrease in the levels of spermidine and spermine that is produced by MGBG may result from an ability of MGBG to accumulate equally well in glial cells in the ganglia which are proliferating postnatally and which would be expected to have higher turnover rates of polyamines [4, 5]. It is possible that guanethidine might inhibit increases in polyamines after a stimulus (e.g. nerve growth factor) which increases ornithine decarboxylase and presumably polyamine biosynthesis.

In summary, guanethidine has been shown to be a potent competitive inhibitor ($K_i = 25\ \mu M$) of SAM DC from several tissues and a moderately potent non-competitive inhibitor of thymus diamine oxidase ($K_i = 90\ \mu M$) in the rat. Polyamine levels, however, are not decreased in tissues (sympathetic ganglia, adrenal medulla) in which guanethidine accumulates to a high concentration. Although it does not appear that the ability to inhibit either of these enzymes explains the cytotoxic effects of the drug on sympathetic neurons, inhibition of these enzymes may be important in other pharmacologic and toxicologic properties of this agent which is widely used clinically and as an experimental tool.

Acknowledgements—The authors would like to thank Glennon Fox and Richard Macia for their assistance.

Department of Pharmacology, Washington University Medical School, St. Louis, MO 63110, *U.S.A.*

EUGENE M. JOHNSON*
ALAN S. TAYLOR

REFERENCES

1. G. Burnstock, B. Evans, B. J. Gannon, J. W. Heath and V. James, *Br. J. Pharmac.* **43**, 295 (1971).
2. P. Juul and J. Jensen-Holm, *Acta pharmac. tox.* **30**, 308 (1971).
3. L. Eränkö,. *Acta pharmac. tox.* **30**, 403 (1971).
4. J. Jänne, H. Pösö and A. Raina, *Biochim. biophys. Acta* **473**, 241 (1978).
5. D. V. Maudsley, *Biochem. Pharmac.* **28**, 153 (1979).
6. E. Hölttä, P. Hannonen, J. Pispa and J. Jänne, *Biochem. J.* **136**, 669 (1973).
7. M. A. Beaven, G. Wilcox and G. K. Torpstra, *Analyt. Biochem.* **84**, 638 (1978).
8. N. Tryding and B. Willert, *Scand. J. clin. Lab. Invest.* **22**, 29 (1968).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
10. T. O. Eloronta and A. M. Raina, *Biochem. biophys. Res. Commun.* **84**, 23 (1978).
11. E. M. Johnson and F. E. Hunter, *Biochem. Pharmac.* **28**, 1525 (1979).
12. P. Juul and O. Sand, *Acta pharmac. tox.* **32**, 487 (1973).
13. E. M. Johnson and F. O'Brien, *J. Pharmac. exp. Ther.* **196**, 53 (1976).
14. I. A. Hendry, *J. Neurocytol.* **6**, 299 (1977).

* An Established Investigator of the American Heart Association.

Biochemical Pharmacology, Vol. 29, pp. 115–118
Pergamon Press Ltd. 1980. Printed in Great Britain.

Quantitative structure–activity relationships for the inhibition of heart and brain cyclic AMP phosphodiesterases by some phenylbutenolides

(*Received* 19 *March* 1979; *accepted* 26 *July* 1979)

Cyclic AMP phosphodiesterase (PDE) is ubiquitously distributed in mammalian tissues [1] and exists under a wide variety of multiple forms. This particularity partly explains the differential sensitivities to drugs of the tissues and makes phosphodiesterase a suitable target for the development of specific new drugs. The therapeutic interest of inhibiting specifically the PDE in a given tissue has been recently emphasized by Weiss and Hait [2]. We previously described a new class of synthetic potent inhibitors of phosphodiesterases acting preferentially on the heart PDE [3], and exhibiting cardiotonic properties. These compounds (Fig. 1) are phenylbutenolides differently substituted in the 4 position of the phenyl ring by complex groups of a large size (AP 10:*R* = Glucose—O—C_6H_4—CO—CH_2—; IP 24:*R* = Glucose—O—C_6H_4—CO—NH—; IP 17:*R* = HOOC—CH_2—O—C_6H_4—CO—CH_2—). The present study considers more simply substituted phenylbutenolides (Table 1) synthetized in our laboratory by Prigent *et al.* [4, 5]. The aim of this report was to answer the following questions. (i) Is the presence of a complex aromatic group in the 4 position a prerequisite for the inhibition of PDE? (ii) Does the nature of substituent *R*