Putrescine, a source of γ -aminobutyric acid in the adrenal gland of the rat

Philip C. CARON, Lucien J. COTE* and Leon T. KREMZNER

Departments of Pathology, Rehabilitation Medicine, and Neurology, College of Physicians and Surgeons, Columbia University, New York, NY 10032, U.S.A.

Putrescine is the major source of γ -aminobutyric acid (GABA) in the rat adrenal gland. Diamine oxidase, and not monoamine oxidase, is essential for GABA formation from putrescine in the adrenal gland. Aminoguanidine, a diamine oxidase inhibitor, decreases the GABA concentration in the adrenal gland by more than 70 % after 4 h, and almost to zero in 24 h. Studies using [14 C]putrescine confirm that [14 C]GABA is the major metabolite of putrescine in the adrenal gland. Inhibition of GABA transaminase by amino-oxyacetic acid does not change the GABA concentration in the adrenal gland, as compared with the brain, where the GABA concentration rises. With aminoguanidine, the turnover time of GABA originating from putrescine in the adrenal gland is 5.6 h, reflecting a slower rate of GABA metabolism compared with the brain. Since GABA in the adrenal gland is almost exclusively derived from putrescine, the role of GABA may relate to the role of putrescine as a growth factor and regulator of cell metabolism.

INTRODUCTION

Putrescine (1,4-diaminobutane) is a precursor of the polyamines spermidine and spermine, which are found throughout nature and are involved in numerous cellular regulatory processes (Heby, 1981; Grillo, 1985). However, putrescine also is metabolized to γ -aminobutyric acid (GABA) in mammalian organs under a variety of physiological and developmental conditions (Seiler *et al.*, 1971; Kremzner *et al.*, 1975; DeMello *et al.*, 1976; Sobue & Nakajima, 1978; Sourkes & Missala, 1981; Fogel *et al.*, 1982).

The finding of non-neural GABA in a variety of tissues (Tanaka, 1985) has led to a search for its function outside the nervous system. Emphasis has focused on GABA in the pancreas (Gerber & Hare, 1979; Taniguchi *et al.*, 1979; Vincent *et al.*, 1983; Fung *et al.*, 1985; Caron *et al.*, 1987) and the female reproductive system (Martin del Rio, 1981; Apud *et al.*, 1984; Murashima & Kato, 1986; Celotti *et al.*, 1986).

GABA is present in the adrenal gland (Martin del Rio & Caballero, 1980; Tanaka, 1985), a complex organ with endocrine and neural functions. Biochemical studies have demonstrated the presence of GABA in adrenal chromaffin cells, where there is evidence for its role in modulating the release of catecholamines from the adrenal medulla (Kataoka *et al.*, 1984). Reports that the action of GABA in the adrenal chromaffin cell can be modulated by benzodiazepines (Bormann & Clapham, 1985; Kataoka *et al.*, 1984) have wide implications for the role of GABA in the autonomic nervous system. The fact that GABA and polyamine metabolism is closely associated with nervous and endocrine tissues (Seiler, 1980) makes the adrenal gland an attractive candidate for the study of GABA metabolism outside the central nervous system.

In this paper, we demonstrate the formation of GABA from putrescine, its metabolism *in vivo* and turnover rate in the rat adrenal gland.

MATERIALS AND METHODS

Chemicals

[1,4- 14 C]Putrescine dihydrochloride (102 mCi/mmol) and Aquasol-2 scintillant were obtained from New England Nuclear (Boston, MA, U.S.A.). [14 C]GABA (15 mCi/mmol), putrescine dihydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride and amino-oxyacetic acid hemihydrochloride were obtained from Calbiochem-Behring Corp. (La Jolla, CA, U.S.A.). GABA, 1,7-diaminoheptane, L-proline, L-histidine, cadaverine dihydrochloride, dansyl chloride, dansyl-spermidine, *o*-phthaldialdehyde, Brij-35 and β -mercaptoethanol were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

L-Glutamic acid, 2-aminoethylisothiuronium bromide, phenethylamine and pyridoxal phosphate were also purchased from Sigma; pargyline hydrochloride was from Abbott Laboratories (North Chicago, IL, U.S.A.); lithium citrate and LiOH were from Matheson, Coleman and Bell (Norwood, OH, U.S.A.); aminoguanidine sulphate and heptane-1-sulphonic acid (sodium salt) were from Eastman Kodak Co. (Rochester, NY, U.S.A.). Reserpine, containing ascorbic acid, was obtained from CIBA (Summit, NJ, U.S.A.).

All h.p.l.c. solvents used were h.p.l.c. grade, and were purchased from Waters Associates (Milford, MA, U.S.A.) and Rainin Instrument Co. (Woburn, MA, U.S.A.). Buffers were made in Nanopure water (Barnstead, CO, U.S.A.). Radioactive samples were counted in a Packard Tri-Carb counter. The counting efficiency was 40 % for 3 H and 80 % for 14 C radioactivity.

Animals

Female Sprague-Dawley white rats (150–200 g), obtained from Camm Research Farm (Wayne, NJ, U.S.A.) were used for all experiments. All animals were maintained on a standard rat chow diet and a 12 h-light/12 h-dark cycle.

Abbreviations used: GABA, γ -aminobutyric acid; GAD, glutamate decarboxylase.

* To whom reprint requests should be addressed, at: Department of Rehabilitation Medicine, College of Physicians and Surgeons of Columbia University, 630 West 168th Street, New York, NY 10032, U.S.A.

Table 1. Polyamine and GABA contents in rat brain and adrenal gland

Each value is the mean $\pm$ S.D. for 6–15 brains and adrenal glands. Polyamines and GABA were measured by h.p.l.c. and fluorescent detection as described in the Materials and methods section. 'Brain' excludes brain stem and cerebellum.

Tissue	Content (nmol/g wet wt.)				Spermidine Spermine
	Putrescine	GABA	Spermidine	Spermine	
Brain	3.4 $\pm$ 1.0	2680 $\pm$ 298	173 $\pm$ 31	163 $\pm$ 27	1.1 $\pm$ 0.1
Adrenal gland	7.6 $\pm$ 2.6	29 $\pm$ 8	137 $\pm$ 29	729 $\pm$ 94	0.2 $\pm$ 0.03

Preparation of tissue

In all experiments the animals were killed by decapitation, and the adrenal glands and brain were dissected, and immediately placed in cold 0.9% NaCl, blotted, and weighed. The tissues were homogenized [1:15 (w/v) for adrenal gland; 1:5 (w/v) for brain] in ice-cold 0.4 M-HClO₄ with a motor-driven glass pestle. All tissue extracts were left on ice for at least 30 min, and then were centrifuged at 35 000 *g* for 10 min, in a Sorvall Superspeed RC-2 centrifuge. The pellet was discarded and the supernatant was stored at 4 °C.

Measurement of polyamines and GABA by h.p.l.c.

The polyamines were dansylated and separated by h.p.l.c. on a C₁₈ reverse-phase column (Waters Associates), and were quantified by fluorimetry by methods described by Caron *et al.* (1987). GABA was measured on a h.p.l.c. cation-exchange Aminex A-9 column (Bio-Rad, Richmond, CA, U.S.A.) and quantified by using *o*-phthalaldehyde as described by Caron *et al.* (1987). Standard solutions of putrescine, spermidine and spermine showed linearity with respect to the concentration. The sensitivity was 5–10 pmol, with less than 10% error.

Glutamate decarboxylase (GAD) assay

GAD (L-glutamate 1-carboxy-lyase, EC 4.1.1.15) activity was measured as described by Wu *et al.* (1973), with minor modifications. Freshly dissected adrenal glands and brain were homogenized in 0.05 M-potassium phosphate buffer, pH 6.8, and diluted appropriately for the GAD assay. The reaction mixture (final volume 30 μ l) contained 10 μ l of homogenate (1.5 mg of tissue), 0.1 M-potassium phosphate, pH 7.0, 0.5 mM-pyridoxyl phosphate, 50 mM-L-glutamic acid, and 0.33 μ Ci of DL-[1-carboxy-¹⁴C]glutamic acid (5.34 mCi/mmol). Results were expressed as μ mol of CO₂ formed/h per g wet wt. of tissue.

Statistics

Student's *t* test for unpaired observations and the Pearson product-moment correlation were performed by using the statistical package provided with the Digital MINC-23 laboratory computer. Linear-regression analysis and other standard statistical parameters were determined with a Texas Instruments SR 52 programmable calculator. The 0.05 level of probability was taken as significant.

RESULTS

Endogenous concentrations of polyamines and GABA in rat adrenal gland

The GABA concentration in the adrenal gland is about 4 times that of putrescine, whereas in the brain it is about 800 times that of putrescine (Table 1). The brain contains nearly equivalent concentrations of spermidine and spermine. In the adrenal gland, spermine content is more than 5 times that of spermidine. To assess whether GABA is localized to the cortex or medulla of the rat adrenal gland, the two regions were separated by careful dissection and examined histologically to verify the anatomy. The cortex and medulla of the rat adrenal gland contain 11.7 and 20.6 nmol of GABA/g wet wt. respectively.

GAD activity

The GAD activity in the rat adrenal gland is 2.1 $\pm$ 0.4 μ mol/h per g wet wt., whereas in the whole rat brain it is 19.0 $\pm$ 2.0 μ mol/h per g wet wt. Thus approximately a 10-fold greater GAD activity, and a 100-fold higher GABA concentration, are found in the rat brain as compared with the adrenal gland.

Fig. 1. Metabolism of [¹⁴C]putrescine in the rat adrenal gland as a function of time

[¹⁴C]Putrescine (10 μ Ci, 104 nmol) was injected intraperitoneally in a total volume of 0.2 ml of 0.1 M-sodium phosphate buffer/0.9% NaCl, pH 7.4. The animals were killed at 5, 15, 30, 60 and 120 min after injection. The adrenal glands were dissected and analysed for total radioactivity and radioactive metabolites by h.p.l.c., as described in the Materials and methods section. Key: ▨, putrescine; ■, GABA; *, GABA metabolites; **, spermidine.

Table 2. Specific radioactivity of [^{14}C]GABA after intraperitoneal injection of [^{14}C]putrescine

Values represent means $\pm$ S.D. (* $P < 0.01$); three to five animals were used per determination. [^{14}C]Putrescine (5 μCi) was injected intraperitoneally and the tissues were dissected 30 min later and analysed for both radioactive and endogenous GABA values as described in the Materials and methods section. 'Brain' excludes brain stem and cerebellum.

Organ	$10^{-2} \times [^{14}\text{C}]\text{GABA}$ radioactivity (c.p.m./g wet wt.)	Endogenous GABA (nmol/g wet wt.)	Sp. radioactivity of [^{14}C]GABA (c.p.m./nmol of GABA)
Brain	7 ± 2	2680 ± 298	0.3 ± 0.1
Adrenal	$571 \pm 176^*$	$14 \pm 6^*$	$3771 \pm 864^*$

[^{14}C]Putrescine metabolism in the rat adrenal gland *in vivo*

To determine the distribution and rate of metabolism of putrescine, [^{14}C]putrescine (10 μCi) was injected intraperitoneally, and acid extracts of adrenal glands were fractionated for radioactive metabolites. Approx. 0.05% of the total radioactivity is found in the adrenal glands 30 min after [^{14}C]putrescine is injected. [^{14}C]GABA is virtually the sole metabolite present at every time period in the adrenal gland (Fig. 1). As early as 5 min after [^{14}C]putrescine injection, more than 75% of the total radioactivity in the adrenal gland is [^{14}C]GABA. A trace amount of [^{14}C]spermidine is also detected in the adrenal gland by 1 h.

Specific radioactivity of [^{14}C]GABA in the adrenal gland and brain

The relative contribution of [^{14}C]putrescine to the endogenous pool of GABA in the rat adrenal gland, i.e. [^{14}C]GABA c.p.m./nmol of GABA, is at least 10^4 -fold greater than in the brain (Table 2).

Effects of diamine oxidase and monoamine oxidase inhibitors

Aminoguanidine sulphate (60 mg/kg) and pargyline hydrochloride (60 mg/kg), inhibitors of diamine oxidase (EC 1.4.3.6) and monoamine oxidase (EC 1.4.3.4) respectively, were administered *in vivo* to distinguish between the two possible pathways of GABA formation from putrescine. Aminoguanidine decreases GABA by 70% in the adrenal gland, whereas pargyline does not change the GABA content 4 h after administration. Aminoguanidine, but not pargyline, given 4 h before [^{14}C]putrescine injection, virtually eliminates the formation of [^{14}C]GABA.

Turnover of GABA in the adrenal gland

The effect of aminoguanidine on GABA content in the adrenal gland was investigated in an effort to determine the turnover rate of GABA. The turnover rate is defined here as the time needed to decrease GABA to zero, assuming that it is synthesized and metabolized at a steady rate.

The rate of disappearance of GABA in the adrenal gland is plotted as a function of time after the administration of aminoguanidine (60 mg/kg) (Fig. 2). Aminoguanidine depletes the endogenous GABA content of the adrenal gland to nearly zero in 24 h. Linear-regression analysis shows that the concentration of

Fig. 2. Determination of the turnover time of GABA in the rat adrenal gland

Aminoguanidine sulphate (60 mg/kg) in 0.1 M-sodium phosphate buffer/0.9% NaCl, pH 7.4, was injected intraperitoneally and the animals were killed 15, 30, 60, 90, 120, 240 and 1440 min later. Each value represents mean $\pm$ S.D.; n = three to six animals per determination.

GABA in the adrenal gland declines at a rate of 4.3 nmol/h per g wet wt. Thus the turnover time for GABA is 5.6 h.

Metabolism of GABA in the adrenal gland *in vivo*

Amino-oxyacetic acid hemihydrochloride (25 mg/kg), an inhibitor of GABA transaminase (γ -aminobutyrate:2-oxyglutarate aminotransferase, EC 2.6.1.19), does not significantly change the GABA concentration in the adrenal gland (control, 24 ± 10 nmol/g wet wt.) compared with 4 h after administration (23 ± 10 nmol/g wet wt.).

DISCUSSION

Our studies show that GABA in the rat adrenal gland is derived mainly from putrescine, and not from glutamic acid. Putrescine in the adrenal gland is more readily converted via diamine oxidase into GABA, rather than into the polyamines spermidine and spermine. Since aminoguanidine, a diamine oxidase inhibitor, and not pargyline, a monoamine oxidase inhibitor, drastically decreases the GABA concentration in the adrenal gland, monoamine oxidase cannot play a major role in GABA formation from putrescine.

Using immunohistochemical staining, Kataoka *et al.* (1984) found that 40% of bovine adrenal chromaffin

cells in culture stain positively for GAD, and 70% for GABA transaminase. In light of the new evidence presented in our paper, the significance of GAD-containing cells must be modified and re-examined to include putrescine as a major contributor to GABA in the adrenal gland.

On the basis of our results, the catabolism of GABA in the adrenal gland is similar to that in the rat ovary and oviduct, where amino-oxyacetate does not increase GABA (Erdo *et al.*, 1982; Martin del Rio & Lopez, 1983). This is in contrast with the brain (Perry & Hansen, 1973; Bernasconi *et al.*, 1982) and pancreas (Okada & Taniguchi, 1980; Caron *et al.*, 1987), where GABA is markedly increased after amino-oxyacetate administration.

Data from our studies of [^{14}C]putrescine injections show that the [^{14}C]GABA that is formed in the adrenal gland is not rapidly metabolized. However, in the brain, [^{14}C]putrescine is rapidly metabolized (Caron *et al.*, 1987). This adds further evidence that a different rate of GABA metabolism exists in the two organs.

The turnover time of GABA in the adrenal gland was found to be 5.6 h (Fig. 2). This differs from the short turnover time of 1 h in the rodent brain (Caron *et al.*, 1987). Since aminoguanidine is used in our study, the turnover time reflects the majority of GABA originating from putrescine in the adrenal gland. Whether GAD-derived GABA has a different turnover time from putrescine-derived GABA is yet to be determined.

The strongest evidence for a functional role for GABA in the adrenal gland comes from studies done in the adrenal medulla, where a high uptake and release mechanism for GABA is found in the brain (Sangiah *et al.*, 1974; Yamanaka *et al.*, 1983; Kataoka *et al.*, 1984; Bormann & Clapham, 1985). GABA is known to cause the release of catecholamines from the adrenal medulla, similar to that of autonomic ganglia. This action can be affected by GABA antagonists, such as bicuculline and picrotoxin (Sangiah *et al.*, 1974; Yamanaka *et al.*, 1983).

Further studies of GABA in the adrenal gland must take into consideration its formation from putrescine. In the adrenal gland, GABA is derived almost exclusively from putrescine, a growth factor. This suggests that GABA may play a role in the growth and metabolism of the adrenal gland.

This project was supported by the J.M. Foundation and the Vidda Foundation in the Department of Rehabilitation Medicine, Columbia University. We thank Dr. John Downey and Dr. Henry Vogel for their interest in this project. We are grateful to Sally Ng for her excellent technical assistance and to Anne Winberry for manuscript preparation.

REFERENCES

- Apud, J. A., Tappaz, M. L., Celotti, F., Negri-Cesi, P., Masotto, C. & Racagni, G. (1984) *J. Neurochem.* **43**, 120–125
- Bernasconi, R., Maitre, L., Martin, P. & Raschdorf, F. (1982) *J. Neurochem.* **38**, 57–66
- Bormann, J. & Clapham, D. E. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 2168–2172
- Caron, P. C., Kremzner, L. T. & Cote, L. J. (1987) *Neurochem. Int.* **10**, 219–229
- Celotti, F., Apud, J. A., Melcangi, R. C., Masotto, C., Tappaz, M. & Racagni, G. (1986) *Endocrinology (Baltimore)* **118**, 334–339
- DeMello, F. G., Bachrach, V. & Nirenberg, M. (1976) *J. Neurochem.* **27**, 847–851
- Erdo, S. L., Rosdy, B. & Szporny, L. (1982) *J. Neurochem.* **38**, 1174–1176
- Fogel, W. A., Bieganski, T. & Maslinski, C. (1982) *Comp. Biochem. Physiol.* **73C**, 431–434
- Fung, K. P., Lee, K. W. & Choy, Y. M. (1985) *Nutr. Rep. Int.* **32**, 1267–1271
- Gerber, J. C. & Hare, T. A. (1979) *Diabetes* **28**, 1073–1076
- Grillo, M. A. (1985) *Int. J. Biochem.* **17**, 943–948
- Heby, O. (1981) *Differentiation* **19**, 1–20
- Kataoka, Y., Gutman, Y., Guidotti, A., Panula, P., Wroblewski, J., Cosenza-Murphy, D., Wu, J. Y. & Costa, E. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3218–3222
- Kremzner, L. T., Hiller, J. M. & Simon, E. J. (1975) *J. Neurochem.* **25**, 889–894
- Martin del Rio, R. (1981) *J. Biol. Chem.* **256**, 9816–9819
- Martin del Rio, R. & Caballero, A. L. (1980) *J. Neurochem.* **34**, 1584–1586
- Martin del Rio, R. & Lopez, M. S. (1983) *Gen. Pharmacol.* **14**, 281–283
- Murashima, Y. L. & Kato, T. (1986) *J. Neurochem.* **46**, 166–172
- Okada, Y. & Taniguchi, H. (1980) *Proc. Jpn. Acad. Ser. B* **56**, 46–50
- Perry, T. L. & Hansen, S. (1973) *J. Neurochem.* **21**, 1167–1175
- Sangiah, S., Borowitz, J. L. & Yim, G. K. W. (1974) *Eur. J. Pharmacol.* **27**, 130–135
- Seiler, N. (1980) *Physiol. Chem. Physics* **12**, 411–429
- Seiler, N., Wiechmann, M., Fischer, H. A. & Werner, G. (1971) *Brain Res.* **28**, 317–325
- Sobue, K. & Nakajima, T. (1978) *J. Neurochem.* **30**, 277–279
- Sourkes, T. L. & Missala, K. (1981) *Agents Actions* **11**, 20–27
- Tanaka, C. (1985) *Life Sci.* **37**, 2221–2235
- Taniguchi, H., Okada, Y., Seguchi, H., Shimada, C., Seki, M., Tsutou, A. & Baba, S. (1979) *Diabetes* **28**, 629–633
- Vincent, S. R., Hokfelt, T., Wu, J.-Y., Elde, R. P., Morgan, L. M. & Kimmel, J. R. (1983) *Neuroendocrinology* **36**, 197–204
- Wu, J.-Y., Matsuda, T. & Roberts, E. (1973) *J. Biol. Chem.* **248**, 3029–3034
- Yamanaka, K., Yamada, S., Okada, T. & Hayashi, E. (1983) *Jpn. J. Pharmacol.* **33**, 1049–1055