Biochemical Pharmacology, Vol. 37, No. 19, pp. 3613–3618, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

REDUCED AMOUNTS OF *S*-ADENOSYLMETHIONINE DECARBOXYLASE IN THE ADRENAL GLANDS OF RATS FOLLOWING ADMINISTRATION OF PIRIBEDIL OR 2-DEOXYGLUCOSE

MARC EKKER,* THEODORE L. SOURKES† and RON GABOR
Departments of Biochemistry and Psychiatry, McGill University, Faculty of Medicine, Montreal, Canada

(*Received* 14 *December* 1987; *accepted* 7 *March* 1988)

Abstract—The activity of *S*-adenosylmethionine decarboxylase (SAM-DC) is decreased in the adrenal gland of the rat following physical stress, metabolic stress or administration of dopamine agonists [M. Ekker and T. L. Sourkes, *Endocrinology* **120**, 1299 (1987)]. Immunotitration studies with a serum directed against purified rat liver SAM-DC show that the reduction in activity of the enzyme following administration of 2-deoxyglucose or piribedil was paralleled by a decrease in the amount of immunoreactive protein. There was no difference in the half-life of SAM-DC activity between piribedil-treated rats and controls. The properties of an extensively purified preparation of the adrenal enzyme resembled those of SAM-DC obtained from rat liver. It is suggested that the reduction in adrenal SAM-DC activity and protein content caused by stress is due to a reduction in the rate of synthesis of the enzyme.

S-Adenosylmethionine decarboxylase (EC 4.1.1.50, SAM-DC) catalyses the second step in the biosynthesis of polyamines. Along with ornithine decarboxylase (EC 4.1.1.17, ODC), it plays a regulatory role in the formation of spermidine and spermine. SAM-DC is regulated in a dual manner in response to stimuli associated with growth. First, it is activated by putrescine [1], and a greater availability of this diamine as a consequence of ODC induction by growth-associated stimuli is thought to result in increased activity of SAM-DC. Moreover, increased amounts of the enzyme protein have been observed in response to some of the stimuli that cause the induction of ODC [2]. Rapid regulation of the enzyme content is made possible partly because of the short half-life of SAM-DC [3].

We have shown that the activity of SAM-DC in the adrenal gland is decreased in response to treatments that cause adrenal ODC activity to increase. These treatments include: the stress of immobilization, administration of 2-deoxyglucose and insulin which provide metabolic stress, and the administration of dopamine agonists such as apomorphine and piribedil [4]. These decreases in SAM-DC activity which apply to both the adrenal medulla and cortex depend upon the availability of corticotropin (ACTH). The present work is an attempt to elucidate the mechanism by which the treatments mentioned above bring about a decrease in SAM-DC activity. We examined by immunotitration the changes in SAM-DC enzyme content following administration of piribedil or of 2-deoxyglucose, and determined the half-life of the enzyme in adrenal glands of untreated rats (controls) and of rats receiving piribedil. We also studied some properties of adrenal SAM-DC in crude and partially purified preparations.

MATERIALS AND METHODS

Materials. *S*-Adenosyl-L-[*carboxyl*-^{14}C]methionine, specific activity 50–60 Ci/mol, was purchased from New England Nuclear (Boston, MA). Piribedil [1-(3,4-methylenedioxybenzyl)-4-(2-pyrimidyl)-piperazine] was received as a gift from Laboratoires Servier (Neuilly-sur-Seine, France). 6-Aminohexanoic acid-Sepharose 4B was purchased from Pharmacia Fine Chemicals (Canada) Ltd. (Dorval, Quebec). *S*-Adenosylmethionine, methylglyoxal bis-guanylhydrazone, putrescine, dithiothreitol, sodium deoxycholate, cycloheximide and 2-deoxyglucose were purchased from the Sigma Chemical Co. (St. Louis, MO).

Animals. Male Sprague–Dawley rats, weighing about 200 g, were used throughout this work except for the purification of SAM-DC where animals weighing 420–480 g were used. They were purchased from Canadian Breeding Farms and Laboratories (St. Constant, Quebec).

Drug treatments. Piribedil was suspended in 1% methylcellulose and given subcutaneously in a dose of 50 mg/kg (volume of injection: 2.5 ml/kg). All other drugs were dissolved in saline and given subcutaneously in the following doses: 2-deoxyglucose, 500 mg/kg; cycloheximide, 10 mg/kg; methylglyoxal bis-guanylhydrazone (MGBG), 80 mg/kg.

Tissue preparation. The rats were killed by

* Present address: Dépt. d'Immunologie, Institut Pasteur, 75724 Paris CEDEX 15, France.
† Address for correspondence and reprints: Dr. T. L. Sourkes, Department of Psychiatry, McGill University, 1033 Pine Ave. West, Montreal, Quebec, Canada H3A 1A1.

decapitation. The adrenals were quickly removed, cooled on ice, freed of capsular tissue and weighed. Two glands were homogenized in 200 μl of 0.05 M sodium–potassium phosphate buffer, pH 7.2, unless otherwise mentioned.

Adenosylmethionine decarboxylase assay. The activity of SAM-DC was determined by measuring the production of $^{14}CO_2$ from *S*-adenosyl-L-[*carboxyl*-^{14}C]methionine, as described by Pegg and Williams-Ashman [1], with some modifications. The reaction mixture contained (final concentrations given): sodium–potassium phosphate buffer, 0.05 M, pH 7.2; putrescine, 2.5 mM; dithiothreitol, 1 mM; EDTA, 0.1 mM; 0.4 μCi of *S*-adenosyl-L-[*carboxyl*-^{14}C]methionine, 0.1 M; and 100 μl of the 105,000 *g* supernatant fraction of the homogenate (centrifugation time: 30 min), in a total volume of 0.5 ml. One unit of enzyme activity was defined as the amount of enzyme producing 1 nmol CO_2 per min of incubation at 37°.

Protein content of the different enzyme fractions was determined by the method of Lowry *et al.* [5].

Enzyme purification. *S*-Adenosylmethionine decarboxylase was purified from the liver and adrenals of fifty-one Sprague–Dawley rats weighing 420–480 g. These animals received a single injection of MGBG 24 hr before death in order to increase the amount of enzyme [6]. The purification of the liver enzyme was performed according to Sakai and collaborators [7] with minor modifications. A summary of the purification of rat liver SAM-DC is shown in Table 1. The final preparation was analysed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). It gave one major band with a molecular weight of 32,000 daltons (Fig. 1A). This preparation was used for the immunization of rabbits in order to obtain an antibody against SAM-DC.

The purification of the adrenal enzyme on DEAE-cellulose and MGBG-Sepharose columns was performed in a way identical to the purification of the liver enzyme except for the following points: (1) a small column of DEAE-cellulose (1.6 × 25 cm) was utilized, and (2) only one chromatographic separation on MGBG-Sepharose was performed and the purified enzyme was kept in elution buffer with added sodium deoxycholate (0.1%). The chromatographic separation on Sephacryl S-200 was omitted. A summary of the purification of the adrenal enzyme is shown in Table 1.

SDS-PAGE was performed according to Laemmli [8], and the gels were stained with the Bio-Rad silver stain kit (Bio-Rad Laboratories Canada Ltd., Mississauga, Ontario).

Immunization with liver SAM-DC. Antibodies to rat liver SAM-DC were raised in a New Zealand white female rabbit. Five hundred micrograms of the enzyme purified from rat liver was emulsified in Freund's complete adjuvant, and the rabbit was injected subcutaneously at multiple sites along the back. A control rabbit received an equivalent injection of 500 μg of bovine serum albumin. Booster injections (200 μg of purified enzyme emulsified in Freund's incomplete adjuvant) were given 14, 26, and 36 days and 6 months after the first injection. A blood sample was taken from the rabbits 11 days after the third and the last booster injections; partially purified immunoglobulin fractions were prepared by precipitation at 40% saturation with ammonium sulfate [9].

Immunotitration of adrenal SAM-DC. Immunotitration, with rabbit anti-rat SAM-DC, was performed in polypropylene conical centrifuge tubes. Each tube contained the supernatant fraction of the adrenal gland homogenate (105,000 *g*; 30 min) and the antiserum in a total volume of 150 μl of a solution that contained: sodium–potassium phosphate buffer, pH 7.5, 25 mM; dithiothreitol, 1 mM; EDTA, 0.1 mM; putrescine, 2.5 mM; and NaCl, 15 mM. The tubes were incubated for 16–20 hr at 4° and then centrifuged for 15 min at 12,000 *g*. The enzyme activity was determined in 100-μl aliquots of the supernatant fraction.

The incubations generally contained 400 μg of adrenal extract protein and various amounts (0–1 μl) of the antiserum.

Table 1. Purification of rat liver and adrenal *S*-adenosylmethionine decarboxylase

Fraction	Purification step	Total protein (mg)	Total units	Specific activity (units/mg)	Purification (X)	Yield (%)
(A) Liver						
1	Crude supernatant	23,229	689	0.03	1	100
2	Ammonium sulfate	11,928	566	0.05	1.6	82
3	DEAE-cellulose	455	223	0.49	16	32
4	1st MGBG-Sepharose	1.3	128	98.5	3283	19
5	2nd MGBG-Sepharose	0.65	86	132.2	4410	12
6	Sephacryl S-200	0.16	45	288.5	9617	7
(B) Adrenal						
1	Crude supernatant	223	7.74	0.035	1	100
2	Ammonium sulfate	157	9.30	0.059	1.7	120
3	DEAE-cellulose	4.3	4.36	1.02	29	56
4	MGBG-Sepharose	0.05	4.18	83.6	2389	54

One unit of activity is defined as the amount of enzyme catalysing the production of 1 nmol of CO_2/min at 37°.

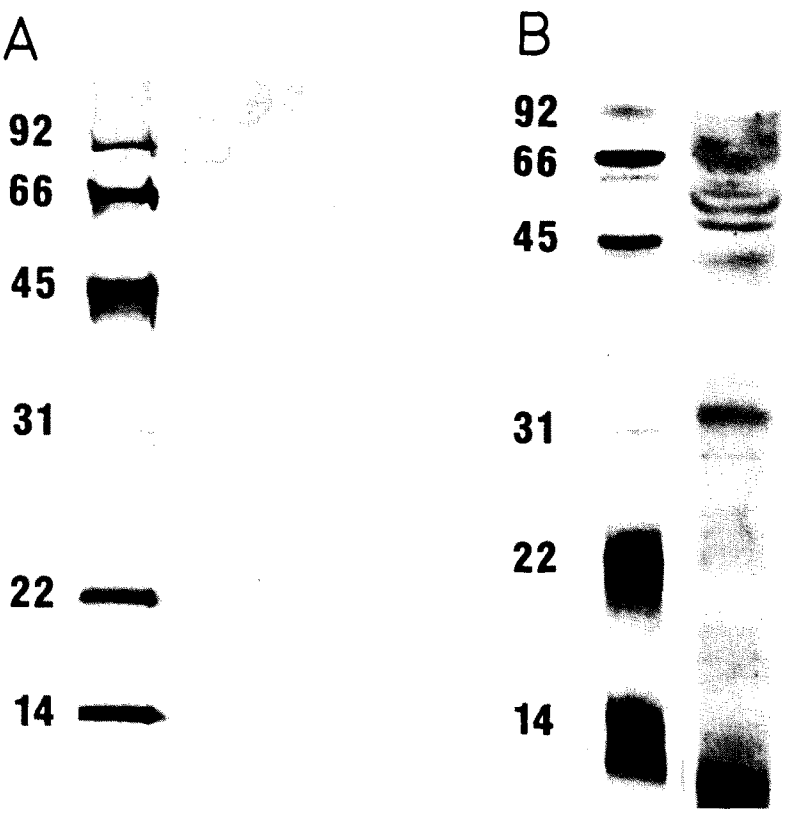

Fig. 1. SDS-PAGE of purified liver and adrenal SAM-DC. (A) Liver enzyme. Left lane: molecular weight markers; right lane: about 100 ng of protein from Fraction 6. (B) Adrenal enzyme. Left lane: molecular weight markers; right lane: about 10 μg of protein from Fraction 4. The gels (12%) were silver-stained.

RESULTS

Effects of piribedil and 2-deoxyglucose on immunoprecipitable SAM-DC activity. A partially purified immunoglobulin fraction prepared from the serum of a rabbit immunized with purified liver SAM-DC (see Fig. 1 and Materials and Methods) was able to precipitate the activity contained in crude enzyme preparations from rat liver or adrenals. One unit of enzyme activity could be precipitated with 3.2 mg of immunoglobulin. The immunoglobulin preparation had a protein concentration of 8.5 mg/ml. A similar immunoglobulin preparation from a control rabbit immunized with bovine serum albumin did not precipitate SAM-DC activity of rat liver or adrenals even when added to the immunotitration assay in ten times larger amounts.

The amount of immunoglobulin preparation inactivating 50% of SAM-DC activity present in about 400 μg of cytosol proteins from adrenal of rats treated with piribedil, 2-deoxyglucose or in control (saline-treated) rats was estimated from immunotitration curves (Fig. 2). The ratio of the enzyme activity of the extract to the amount of antibody required to obtain 50% inactivation was roughly equal in all cases indicating a parallelism between enzyme activity and the amount of enzyme protein estimated by immunotitration. Therefore, it seems that the reduced activity observed in the adrenals of rats

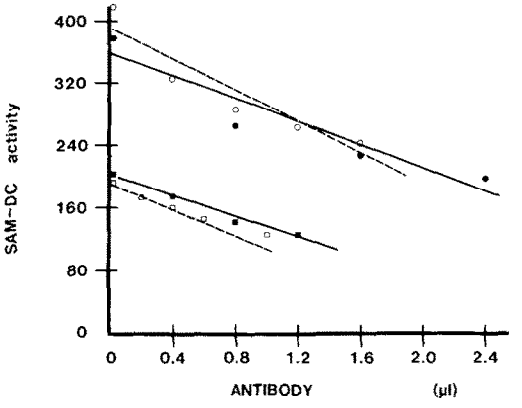

Fig. 2. Immunotitration of adrenal SAM-DC with rabbit anti-rat liver SAM-DC antibody. Each incubation contained 400 μg of cytosol protein. Key: (●—●), controls, Expt. 1; (○—○), controls, Expt. 2; (■—■), piribedil-treated rats (50 mg/kg, Expt. 1); and (□—□), 2-deoxyglucose-treated rats (500 mg/kg, Expt. 2). All rats were killed 4 hr after the injection of drug or saline. A minimum of eight rats was used for each treatment, and their adrenal glands were pooled. SAM-DC is expressed as pmol CO_2/30 min per mg protein. The lines drawn result from regression analysis of the data. Correlation coefficients were: (●) −0.95; (■) −0.98; (○) −0.83; and (□) −0.88. Ratio of enzyme activity to the amount (μl) of antibody needed for 50% inhibition: (●) 144; (■) 134; (○) 202; and (□) 172.

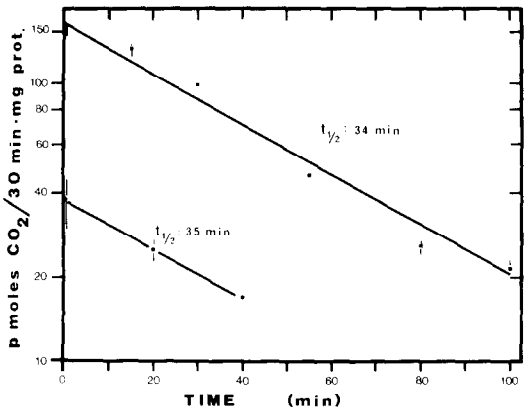

Fig. 3. Effect of cycloheximide on loss of adrenal SAM-DC activity (whole gland). Rats received a single injection of piribedil (50 mg/kg, ●) or saline (■). A minimum of eight to twelve rats was used for each treatment, and their adrenal glands were pooled. Three hours later, they received a single intraperitoneal injection of cycloheximide (10 mg/kg) and were killed at various times thereafter (X-axis). Values are expressed as the mean ± 1 SEM, based upon three separate determinations. Bars represent SEM and are omitted where their magnitude is less than the symbol portraying the mean.

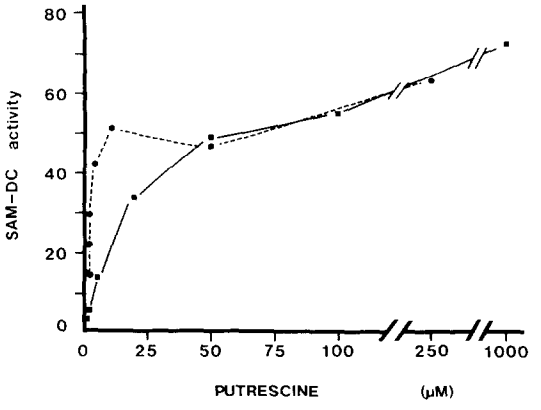

Fig. 4. Adrenal SAM-DC activity as a function of putrescine concentration. Key: (■—■) about 600 μg of crude supernatant fraction of an adrenal homogenate; incubation time: 20 min and (●—●) about 0.2 μg of purified adrenal SAM-DC (Fraction 4); incubation time: 10 min. Enzyme activity is expressed as pmol CO_2/30 min.

receiving piribedil or 2-deoxyglucose is attributable to a reduced enzyme content.

The half-life of adrenal SAM-DC was estimated from the rate of loss of activity following a single injection of cycloheximide (Fig. 3). The rate of loss of activity was identical in control rats and rats that had received an injection of piribedil 3 hr prior to the injection of cycloheximide. The value of the estimated half-life of adrenal SAM-DC activity, about 35 min, is similar to that reported for other rat organs [10, 11].

Properties of adrenal SAM-DC in crude or partially purified preparations. SAM-DC was extensively purified from the adrenals of MGBG-treated rats as described in Materials and Methods. The active fraction obtained after one chromatographic separation on MGBG-Sepharose (Fraction 4) had a specific activity of about 84 units/mg protein with a yield of 54% (Table 1). Fraction 4 was stored in elution buffer containing 0.5 M NaCl and 0.1% sodium deoxycholate to prevent losses in activity that could be caused by the very low concentration of proteins [7]. Dialysis of Fraction 4 against buffer that did not contain NaCl, and reduction of the sample volume by lyophilisation were essential to obtain adequate separation upon SDS-PAGE. Such a separation is shown in Fig. 1B. A major band was observed at 32,000 daltons as well as a diffuse band in the lower molecular weight region which could very well correspond to products of degradation of the enzyme. Other bands were observed especially in the molecular weight region between 66,000 and 90,000 daltons. In a subsequent preparation, when Fraction 4 was subjected to a second chromatographic separation on MGBG-Sepharose, this additional step resulted in nearly total loss of enzyme activity (less than 1% recovery; M. Ekker and T. L. Sourkes, unpublished observation).

The apparent K_m of adrenal SAM-DC for its substrate was estimated by double-reciprocal plot analysis. Values of 24, 30 and 32 μM were obtained for the enzyme Fraction 4 (from MGBG-treated rats), a non-purified cytosolic preparation from non-treated rats, and a similar preparation from piribedil-treated rats respectively.

The activity of adrenal SAM-DC, like the enzyme from other mammalian tissues, was stimulated markedly by putrescine. However, the magnitude of the stimulation as well as the amount of putrescine required to obtain half the maximal stimulation depended upon the degree of purification of the enzyme. The purified enzyme was stimulated to a maximum of 3.5-fold by putrescine with a K_a of about 1 μM (Fig. 4); the enzyme present in the supernatant fraction of the homogenate was stimulated up to 18-fold with a K_a of approximately 20 μM. A similar discrepancy between the activation pattern of purified SAM-DC as compared to less purified fractions has also been reported for the enzyme from mouse liver [7].

DISCUSSION

The activity of SAM-DC in the adrenal gland of the rat is decreased rapidly following stressors or pharmacological treatments that stimulate the hypothalamo-hypophyseal-adrenocortical axis [4]. The results of immunotitration studies presented here suggest that for at least two of these treatments, administration of piribedil or 2-deoxyglucose, reduced activity is paralleled by diminished amounts of the immunoreactive protein. The rate of loss of activity following administration of cycloheximide was identical in control and piribedil-treated rats, thus indicating that reduced SAM-DC content could be due to a change in the rate of its synthesis. We found no evidence for a modification of the protein

that would result in a reduced enzymatic activity. Kinetic analysis of crude adrenal preparations from normal and piribedil-treated rats showed no difference except for V_{max}.

Adrenal SAM-DC was purified extensively, although not to homogeneity. From the values of specific activity of the enzyme in crude and purified fractions we can estimate that SAM-DC represents less than 0.04% of total cytosolic proteins of the adrenal gland. The K_m of the purified enzyme for *S*-adenosylmethionine, 24 μM, which is very similar to that determined in a crude adrenal preparation, was of the same order of magnitude as that reported for the liver enzyme (50 μM; [12]). Tissue concentrations of *S*-adenosylmethionine of 40 μmol/kg have been reported for male Sprague–Dawley rats [13]; this suggests that SAM-DC may not be totally saturated with its substrate *in vivo*. Although increases in adrenal content of *S*-adenosylmethionine have been reported following repeated stress [14], these increases are small (about 10–20%) and would probably not affect SAM-DC activity significantly.

As previously reported for rat liver and psoas muscle, as well as other animal tissues [1, 12, 15, 16], adrenal SAM-DC was stimulated by putrescine. A discrepancy exists between the enzyme from crude extract and the purified enzyme in regard to the magnitude of the stimulation and the K_a associated with it. As mentioned earlier, a similar discrepancy has been noted by Sakai and his colleagues [7] who also observed a much smaller K_a in the most purified preparation of mouse liver SAM-DC as compared to less purified fractions. Pegg and collaborators [2] have suggested that this difference in K_a could be attributed to Sakai's use of a detergent during the course of the purification. In fact, less purified preparations lacking the detergent required greater amounts of putrescine for activation. In our hands, the purified enzyme from rat liver (which was stored in a buffer containing detergent) showed activation kinetics similar to those described by Sakai and collaborators, and these kinetics were not affected if the preparation was dialysed against a buffer from which the detergent was omitted (M. Ekker and T. L. Sourkes, unpublished observations). Therefore, the difference is probably not attributable to the presence of the detergent in the reaction mixture but possibly to a change in the protein induced by the detergent in the course of the purification. It is possible that some other constituent in the crude extract binds putrescine, but it is unlikely that a sufficient amount of the amine would be withdrawn from solution to affect the K_a to the extent seen in Fig. 4.

If we assume that the kinetics of activation by putrescine as described for the crude enzyme preparation are representative of the situation *in vivo*, then changes in the adrenal concentration of putrescine, for instance as a result of stress, could significantly affect the activity of SAM-DC. Concentrations of putrescine for the whole adrenal tissue are of the order of less than 10 μmol/kg in the unstressed animal and can rise up to 100–200 μmol/kg, 6–7 hr after the onset of immobilization stress or of a series of injections of apomorphine, which pharmacologically simulate stress [17]. This postulated increase in activity would therefore operate in a direction opposite to the decreases in activity observed in response to the same treatments when SAM-DC activity is measured in conditions of saturation with putrescine [4].

From the studies presented here, it seems that two mechanisms acting in opposite directions influence the activity of SAM-DC in the adrenal gland of the rat. (a) Increased activity of the enzyme could result from the increase in putrescine concentration that follows stress or administration of dopamine agonists. One must, however, be careful when analysing the effect of such variations in putrescine on the production of decarboxylated *S*-adenosylmethionine because of the lack of definitive evidence for the subcellular localization of both the enzyme and its activator. (b) At the same time, the treatments mentioned above clearly cause the amounts of SAM-DC protein to decrease as shown by the diminished activity in conditions of saturation with its activator and the decreased amounts of immunoprecipitable protein.

A role for putrescine in the adrenal gland of rats under stressful conditions other than as a precursor of the polyamines and regulator of SAM-DC cannot be excluded.

In this work, physiological conditions that result in increased amounts of ODC, the other regulatory enzyme in the formation of spermidine and spermine, at the same time bring about parallel decreases in SAM-DC activity and enzyme protein. To our knowledge, the adrenal gland of the rat is the only mammalian organ for which such changes have been documented as occurring in the opposite directions for ODC and SAM-DC respectively. The control over the activity of SAM-DC seems to be mediated by cyclic AMP [4]. The study of the regulation of the gene(s) coding for SAM-DC in different mammalian tissues should help clarify our understanding of the control of polyamine production.

Acknowledgements—This work was supported by the Medical Research Council of Canada. M. E. held a "Sciences 1967" Studentship of the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

1. A. E. Pegg and H. G. Williams-Ashman, *J. biol. Chem.* **44**, 682 (1969).
2. A. E. Pegg, H. Hibasami, I. Matsui and D. R. Bethell, *Adv. Enzyme Regulat.* **19**, 427 (1981).
3. A. E. Pegg, *J. biol. Chem.* **254**, 3249 (1979).
4. M. Ekker and T. L. Sourkes, *Endocrinology* **120**, 1299 (1987).
5. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
6. A. E. Pegg, *Biochem. J.* **141**, 581 (1974).
7. T. Sakai, C. Hori, K. Kano and T. Oka, *Biochemistry* **12**, 5541 (1979).
8. U. K. Laemmli, *Nature, Lond.* **227**, 680 (1970).
9. R. D. Palmiter, T. Oka and R. T. Schimke, *J. biol. Chem.* **246**, 724 (1971).
10. R. H. Fillingame and D. R. Morris, *Biochemistry* **12**, 4479 (1973).

11. A. E. Pegg, A. Corti and H. G. Williams-Ashman, *Biochem. biophys. Res. Commun.* **52**, 696 (1973).
12. H. Pösö and A. E. Pegg, *Biochemistry* **21**, 3116 (1982).
13. D. L. Wong, E. L. Zager and R. D. Ciaranello, *J. Neurosci.* **6**, 758 (1982).
14. S. J. Brown and P. Q. Harris, *Life Sci.* **34**, 97 (1984).
15. M. J. Feldman, C. C. Lévy and D. H. Russell, *Biochemistry* **11**, 671 (1972).
16. P. Hannonen, J. Jänne and A. Raina, *Biochem. biophys. Res. Commun.* **46**, 341 (1972).
17. M. Ekker and T. L. Sourkes, *Biochem. Pharmac.* **34**, 1315 (1985).