Cyclic AMP-binding capacities and histone kinase activation in subcellular components of neocortical tissue

Differential responses to three neurohumoural agents

Jitendra PATEL, Michael NEWMAN* and Henry McILWAIN†

Department of Biochemistry, Institute of Psychiatry (British Postgraduate Medical Federation, University of London), De Crespigny Park, London SE5 8AF, U.K.

(Received 8 August 1980/Accepted 26 September 1980)

1. Noradrenaline and histamine, when added to superfused guinea-pig cerebral-cortical tissues, increased both cyclic AMP-dependent and -independent histone kinase activities of some, but not of all, subsequently isolated subcellular fractions, and decreased their cyclic [^3H]AMP-binding capacity, which was concluded to be due to an increase in endogenously bound cyclic AMP. 2. Adenosine and 2-chloroadenosine also diminished the cyclic [^3H]AMP-binding capacities, but did not affect the histone kinase activities. 3. DEAE-cellulose chromatography and stability to KCl additions showed that the greater part of the histone kinase of the present preparations corresponded to the type II enzyme [of Corbin, Keely & Park (1975) *J. Biol. Chem.* **250**, 218–225], with a lesser amount of type I activity. Different sites of cyclic AMP accumulation in relation to these or other kinases are considered in interpreting the differential tissue responses to the neurohumoural agents examined.

Cyclic AMP, which is known to be produced in cerebral tissues in response to the neurohumoural agents noradrenaline, histamine and adenosine, is thought to mediate their neural actions by stimulating the phosphorylation of endogenous post-synaptic-membrane proteins (Greengard, 1976; Rodnight, 1979). In previous work from this laboratory (M. Newman, J. Patel & H. McIlwain, unpublished work), electrical stimulation of incubated neocortical tissue was shown to result in a delayed increase in the activation ratio for histone kinase in the derived synaptosomal fraction, accompanied by a decrease in cyclic AMP-binding capacity owing to generation of endogenously bound cyclic AMP. Electrical stimulation or K^+ depolarization liberate several neuroactive agents from cerebral tissues, including those named above (Sattin & Rall, 1970; McIlwain & Snyder, 1970; Pull & McIlwain, 1972; Taylor & Snyder, 1973). We have now added them to superfused cerebral tissues to see whether they mimic the effects of electrical stimulation on the histone kinase and binding capacity of tissue-derived subcellular fractions.

*Present address: Department of Biological Chemistry, Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel.

† Present address: Department of Biochemistry, St. Thomas's Hospital Medical School, London SE1 7EH, U.K.

Experimental

Preparation and incubation of guinea-pig cerebral-cortex slices, subcellular fractionation and determination of cyclic AMP were performed as described previously (Newman & McIlwain, 1978); further characterization of the fractions prepared in this manner has been given by Marchbanks (1975). Each subcellular-fractionation experiment was carried out with material from four slices, pooled to minimize variations owing to uptake of the agents used into specific brain areas. The sucrose solutions used for subcellular fractionation contained 5 mM-3-isobutyl-1-methylxanthine to prevent degradation of cyclic AMP generated in the tissues during the fractionation process and thus maintain the activation status of histone kinase.

Histone kinase and cyclic AMP binding

Total histone kinase was measured by a method based on that of Kuo (1972). To assay media (total volume 200 μl , pH 7.4) containing 60 mM-Tris, 12 mM-magnesium acetate, 0.03 mM-EGTA, 0.1 mM-isobutylmethylxanthine, 200 μg of histone, 5 μM -cyclic AMP and 0.8 mM- $[\text{}^{32}\text{P}]\text{ATP}$, 20 μl of tissue extract adjusted to 10–20 μg of protein was added. This mixture was incubated for 5 min at 30°C. The reaction was stopped by 2.5 ml of 5% (w/v)

trichloroacetic acid containing 0.3 M-NaOH, followed immediately by 100 μ l of bovine serum albumin (10 mg/ml), and 5 min later the mixture was centrifuged at 3000 g for 15 min. The pellet was washed three times with 5% trichloroacetic acid containing 0.1 M-H₂PO₄, and its attached radioactivity measured. The assays were performed in triplicate in both the presence and absence of 5 μ M-cyclic AMP with corresponding tubes lacking histone in duplicate; subtraction of control values contributed to the relatively large s.d. values.

The assay for cyclic AMP-independent histone kinase activity was performed as described above, but in the absence of cyclic AMP and with assay media containing cyclic AMP-dependent protein kinase inhibitor (Walsh *et al.*, 1971) sufficient to inhibit the cyclic AMP-dependent protein kinase activity. The results for histone kinase activity are expressed as activation ratios (see Corbin *et al.*, 1973), giving the ratios of activity in the absence of 5 μ M-cyclic AMP to activity in its presence, both without and with subtraction of the cyclic AMP-independent activity ('total' and 'cyclic AMP-dependent' activity ratios respectively). The cyclic [³H]-AMP-binding capacity was assayed at 0°C at a final cyclic AMP concentration of 0.25 μ M, at which concentration complete saturation of binding sites occurred in the absence of endogenous cyclic AMP.

Exchange of labelled and endogenous bound cyclic AMP was minimal when incubation was carried out at 0°C. All samples were assayed in triplicate, with 'blank' tubes in duplicate containing a 200–400-fold excess of cyclic AMP.

DEAE-cellulose chromatography of cerebral extracts for histone kinase

Guinea-pig brain (approx. 8 g) was homogenized in 24 ml of 0.32 M-sucrose to give a 25% (w/v) homogenate, and centrifuged twice at 100 g for 10 min and once at 10 000 g for 20 min with a Sorvall SS-34 rotor. The pellets in each case were discarded and (NH₄)₂SO₄ was added to the supernatant to give 90% saturation. The precipitate was collected by centrifugation at 10 000 g for 20 min and resuspended in 20 ml of a solution containing 50 mM-Tris, 2 mM-EDTA and 6 mM-2-mercaptoethanol, pH 7.4. After dialysis against 5 litres of the same buffer for 18 h and further centrifugation to remove precipitated material, the supernatant was applied to a column (30 cm $\times$ 1.5 cm) of Whatman DEAE-cellulose DE-52 equilibrated with the same buffer, and a 200 ml linear gradient of 0–0.5 M-NaCl was applied. Fractions (3 ml) were collected at a flow rate of 15 ml/h and desalted on a Sephadex G-25 column (15 cm $\times$ 1.5 cm) before being assayed for protein, histone kinase and cyclic [³H]-AMP-binding capacity.

The relationship between cyclic [³H]-AMP-binding

capacity and histone kinase activity for this preparation was determined by keeping 100 μ g of protein with unlabelled cyclic AMP over the concentration range 0–0.4 μ M for 1 h at 0°C, followed by addition of cyclic [³H]-AMP at a final concentration of 1.07 μ M and keeping for a further 1 h at 0°C before filtration. Histone kinase activity was determined for the same concentration range of unlabelled cyclic AMP with 27 μ g of the purified protein.

Materials

Cyclic [8-³H]-AMP (sp. radioactivity 30 Ci/mmol) and [γ -³²P]-ATP (sp. radioactivity 17 Ci/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Adenosine deaminase was from Boehringer Corp. (London) Ltd., Lewes, East Sussex, U.K., 2-chloroadenosine was from Sigma, Kingston-upon-Thames, Surrey, U.K., and 3-isobutyl-1-methylxanthine was from Aldrich Chemical Co., Milwaukee, WI, U.S.A.

Results

Cyclic nucleotide-binding capacities

The agents examined diminished the capacities of the tissues to bind additional subsequently added cyclic AMP. Adenosine showed the largest action in this respect, decreasing the binding capacity to 25% of its original value (Table 1). With 2-chloroadenosine the fall was to 38% and with histamine to 43%; the diminutions are most readily understandable as being due to the cyclic AMP formed by the added compounds.

Of the tissue components examined, supernatant and synaptosomal fractions were most affected by the added agents (Table 1). With adenosine, their binding capacities fell to 21% and 42% of control values; 2-chloroadenosine and noradrenaline were similarly active, but histamine was less so. The mitochondrial fraction shared the diminution caused by histamine and noradrenaline, but not that caused by adenosine.

Histone kinase activities

The histone kinase activities of several tissue fractions were increased by noradrenaline and by histamine (Tables 2 and 3). The effects were greatest in the mitochondrial, synaptosomal and supernatant fractions, being shown when measured in the presence of the cyclic AMP-specific inhibitor to give cyclic AMP-independent activities, and also when expressed as activity ratios for the total or for the cyclic AMP-dependent enzymes.

Adenosine was, however, without significant

Table 1. *Cyclic AMP-binding capacities of tissues incubated with added neurohumoural agents and of derived fractions*
Tissues were incubated with glucose/bicarbonate saline for 30 min followed by superfusion for 20 min with glucose/bicarbonate saline containing the agents indicated below, before being homogenized in 0.32M-sucrose containing 5 mM-isobutylmethylxanthine. Results are means $\pm$ S.D. for the numbers of observations shown at the head of each column in parentheses.

Tissue preparation	Cyclic AMP bound (pmol/mg of protein) after incubation with:					
	No addition (11)	Adenosine (0.1 mM) (3)	Histamine (0.1 mM) (3)	Noradrenaline (0.1 mM) (4)	Adenosine deaminase (10 μ g/ml) (4)	2-Chloro-adenosine (0.1 mM) (3)
Total homogenate	1.86 $\pm$ 0.74	0.47 $\pm$ 0.13*	0.81 $\pm$ 0.26*	1.34 $\pm$ 0.57	1.52 $\pm$ 0.71	0.71 $\pm$ 0.27*
Nuclear fraction	1.19 $\pm$ 0.44	1.66 $\pm$ 0.48	1.19 $\pm$ 0.35	1.41 $\pm$ 0.33	1.83 $\pm$ 0.52	1.33 $\pm$ 0.22
Mitochondrial fraction	2.09 $\pm$ 0.75	1.82 $\pm$ 0.61	1.04 $\pm$ 0.09*	1.21 $\pm$ 0.13*	2.31 $\pm$ 0.89	1.31 $\pm$ 0.43
Supernatant fraction	1.52 $\pm$ 0.79	0.32 $\pm$ 0.14*	0.64 $\pm$ 0.37	0.52 $\pm$ 0.22*	0.78 $\pm$ 0.52	0.32 $\pm$ 0.27*
Synaptosomal fraction	2.20 $\pm$ 0.62	0.93 $\pm$ 0.66*	1.45 $\pm$ 0.45*	0.96 $\pm$ 0.43*	1.02 $\pm$ 0.39*	1.34 $\pm$ 0.27*

* Significantly ($P < 0.05$) less than control values by Student's t test.

Table 2. *Cyclic AMP-independent histone kinase activity of tissues incubated with added neurohumoural agents and of derived fractions*

Tissues were incubated with glucose/bicarbonate saline for 30 min followed by superfusion for 20 min with glucose/bicarbonate saline containing agents as indicated below before being homogenized in 0.32M-sucrose containing 5 mM-isobutylmethylxanthine. Values for activities are means $\pm$ S.D. for the numbers of observations given in parentheses.

Tissue preparation	Histone phosphorylated (nmol/min per mg of protein) after incubation with:					
	No addition (11)	Adenosine (0.1 mM) (3)	Histamine (0.1 mM) (3)	Noradrenaline (0.1 mM) (4)	Adenosine deaminase (10 μ g/ml) (3)	Inosine (0.1 mM) (3)
Total homogenate	0.83 $\pm$ 0.21	0.91 $\pm$ 0.24	0.76 $\pm$ 0.32	0.95 $\pm$ 0.15	0.95 $\pm$ 0.06	0.75 $\pm$ 0.13
Nuclear fraction	0.57 $\pm$ 0.19	0.66 $\pm$ 0.23	0.65 $\pm$ 0.13	0.77 $\pm$ 0.36	0.98 $\pm$ 0.22*	0.52 $\pm$ 0.10
Mitochondrial fraction	0.65 $\pm$ 0.27	0.91 $\pm$ 0.16	0.83 $\pm$ 0.27	1.15 $\pm$ 0.32*	0.97 $\pm$ 0.26	0.76 $\pm$ 0.17
Supernatant fraction	0.90 $\pm$ 0.15	0.72 $\pm$ 0.13	1.30 $\pm$ 0.28*	1.29 $\pm$ 0.38*	0.63 $\pm$ 0.05	0.80 $\pm$ 0.18
Synaptosomal fraction	0.84 $\pm$ 0.28	1.17 $\pm$ 0.15	1.12 $\pm$ 0.46	1.00 $\pm$ 0.40	1.08 $\pm$ 0.11	0.92 $\pm$ 0.13

* Significantly ($P < 0.02$) greater than control values by Student's t test.

action on histone kinase activities of the different categories just noted, and when expressed in the different fashions indicated (Tables 2 and 3).

Differential actions on binding capacities and histone kinase

The dissociation between the effects of adenosine on histone kinase and on cyclic [3 H]AMP-binding capacity was investigated further by use of adeno-

sine deaminase, which is known to diminish the cyclic AMP of stimulated tissues by removal of extracellular endogenous adenosine (Huang *et al.*, 1973), and of 2-chloroadenosine, an adenosine analogue resistant to the action of the deaminase. Adenosine deaminase increased both cyclic AMP-independent histone kinase and the total histone kinase activation ratios (see the Experimental section) in the nuclear fraction only, but this effect disappeared when cyclic AMP-dependent activity

Table 3. *Cyclic AMP-dependent histone kinase activity of tissues incubated with added neurohumoural agents and of derived fractions*

Tissues were incubated with glucose/bicarbonate saline for 30 min followed by superfusion for 20 min with glucose/bicarbonate saline containing agents as indicated below before being homogenized in 0.32 M-sucrose containing 5 mM-isobutylmethylxanthine. Results are means $\pm$ S.D. for the numbers of observations given in parentheses at the head of each column.

Agent ...	Total kinase activation ratio						
	None (10)	Adenosine (0.1 mM) (3)	Histamine (0.1 mM) (3)	Noradrenaline (0.1 mM) (4)	Adenosine deaminase (10 μ g/ml) (3)	2-Chloro- adenosine (0.1 mM) (4)	Inosine (0.1 mM) (3)
Total homogenate	0.70 $\pm$ 0.13	0.69 $\pm$ 0.06	0.66 $\pm$ 0.02	0.80 $\pm$ 0.10	0.76 $\pm$ 0.02	0.57 $\pm$ 0.17	0.76 $\pm$ 0.06
Nuclear fraction	0.71 $\pm$ 0.15	0.67 $\pm$ 0.02	0.79 $\pm$ 0.15	0.64 $\pm$ 0.12	0.77 $\pm$ 0.07*	0.50 $\pm$ 0.12	0.63 $\pm$ 0.24
Mitochondrial fraction	0.58 $\pm$ 0.12	0.58 $\pm$ 0.10	0.63 $\pm$ 0.11	0.63 $\pm$ 0.10	0.71 $\pm$ 0.06	0.72 $\pm$ 0.05	0.61 $\pm$ 0.13
Supernatant fraction	0.59 $\pm$ 0.14	0.58 $\pm$ 0.10	0.67 $\pm$ 0.06	0.71 $\pm$ 0.10	0.59 $\pm$ 0.08	0.76 $\pm$ 0.11	0.61 $\pm$ 0.06
Synaptosomal fraction	0.66 $\pm$ 0.14	0.71 $\pm$ 0.09	0.94 $\pm$ 0.18*	0.76 $\pm$ 0.18	0.73 $\pm$ 0.06	0.64 $\pm$ 0.09	0.71 $\pm$ 0.10
	Cyclic AMP-dependent kinase activation ratio						
	None (10)	Adenosine (0.1 mM) (3)	Histamine (0.1 mM) (3)	Noradrenaline (0.1 mM) (4)	Adenosine deaminase (10 μ g/ml) (3)	2-Chloro- adenosine (0.1 mM) (4)	Inosine (0.1 mM) (3)
Total homogenate	0.48 $\pm$ 0.17	0.51 $\pm$ 0.04	0.46 $\pm$ 0.16	0.62 $\pm$ 0.08	0.58 $\pm$ 0.03	—	0.47 $\pm$ 0.06
Nuclear fraction	0.37 $\pm$ 0.16	0.49 $\pm$ 0.08	0.62 $\pm$ 0.19*	0.34 $\pm$ 0.13	0.55 $\pm$ 0.09	—	0.46 $\pm$ 0.11
Mitochondrial fraction	0.37 $\pm$ 0.21	0.46 $\pm$ 0.17	0.52 $\pm$ 0.04	0.36 $\pm$ 0.15	0.43 $\pm$ 0.04	—	—
Supernatant fraction	0.43 $\pm$ 0.19	0.36 $\pm$ 0.16	0.46 $\pm$ 0.02	0.55 $\pm$ 0.15	0.52 $\pm$ 0.07	—	0.33 $\pm$ 0.15
Synaptosomal fraction	0.43 $\pm$ 0.14	0.42 $\pm$ 0.21	0.98 $\pm$ 0.37*	0.71 $\pm$ 0.12*	0.27 $\pm$ 0.09	—	0.27 $\pm$ 0.16

* Significantly ($P < 0.05$) greater than control values by Student's t test.

alone was considered (Table 3). The decrease in cyclic [3 H]AMP binding capacity observed in the synaptosomal fraction (Table 1) was not accompanied by any corresponding change in histone kinase activity. Similarly, superfusion with 0.1 mM-2-chloroadenosine, which increased the homogenate cyclic AMP concentrations from 35.3 ± 2.0 to 126.3 ± 19.4 pmol/mg of protein (mean $\pm$ S.D. for three observations in each case) had no effect on cyclic AMP-dependent histone kinase, although the expected decrease in cyclic [3 H]AMP-binding capacity was seen in the homogenate, supernatant and synaptosomal fractions (Table 1). To investigate the possibility that the effects observed with adenosine deaminase might be due to generation of inosine in the tissues, superfusion with 0.1 mM-inosine was performed, but no effects on either histone kinase or cyclic [3 H]AMP-binding capacity were observed.

The nature of the histone kinase enzyme being measured in these experiments was studied by assay of its activity in 0.1 M-KCl and fractionation of the supernatant enzyme by DEAE-cellulose chromato-

graphy. Homogenization of slices incubated in either glucose/bicarbonate saline (Pull & McIlwain, 1972) alone or in the presence of 0.5 mM-histamine with KCl resulted in no significant change in activation ratio, indicating that, unlike the enzyme in heart tissue (Corbin *et al.*, 1975), the cerebral histone kinase now being observed was not dissociated by high ionic strength. The elution profile of an $(\text{NH}_4)_2\text{SO}_4$ extract of the supernatant fraction showed two major peaks of histone kinase activity (Fig. 1). The first, eluted at 0.08 M-NaCl and corresponding to the type I enzyme of Corbin *et al.* (1975), gave an activation ratio for total histone kinase of 0.9. The second, eluted at 0.2 M-NaCl and with an activation ratio of 0.4, represented $83 \pm 5\%$ of the enzyme activity recovered, and corresponded to the type II enzyme of Corbin *et al.* (1975). Both peaks were associated with cyclic [3 H]AMP-binding activity, and the second represented $76 \pm 4\%$ of the total binding activity recovered (mean $\pm$ S.D. for data from four fractionations). Exposure of material from this peak at 0°C to increasing concentrations of unlabelled cyclic AMP (Fig. 2) resulted in a progressive

Fig. 1. DEAE-cellulose chromatography of guinea-pig cerebral-cortex supernatant

Sample preparation and analysis of the fractions were as described in the Experimental section. —, NaCl concentration; ●, cyclic [^3H]AMP-binding capacity, measured at a final cyclic [^3H]AMP concentration of $0.26\ \mu\text{M}$; Δ , histone kinase activity measured in the absence of added cyclic AMP; $\circ$, histone kinase activity measured in the presence of added $1\ \mu\text{M}$ -cyclic AMP. All fractions were assayed in duplicate with one histone blank tube for each condition.

Fig. 2. Relationship between cyclic [^3H]AMP-binding capacity and histone kinase activity for purified histone kinase

Material from the second peak eluted from the column shown in Fig. 1 was desalted and kept with various concentrations of unlabelled cyclic AMP for 1 h at 0°C before addition of $1.07\ \mu\text{M}$ -cyclic [^3H]AMP for determination of cyclic [^3H]AMP-binding capacity (●). Histone kinase activity was assayed over the same range of cyclic AMP concentrations (○).

decrease in cyclic [^3H]AMP-binding capacity measured at a final concentration of $1.07\ \mu\text{M}$, concomitant with an increase in histone kinase activity that reached a plateau at $0.15\ \mu\text{M}$.

Discussion

The present work has shown that noradrenaline and histamine cause an increase in cyclic AMP-dependent histone kinase and a simultaneous decrease in cyclic [^3H]AMP-binding capacity. That this latter effect is due to increased intracellular concentrations of endogenously bound cyclic AMP was first suggested by the work of Do Khac *et al.* (1973), with rat diaphragms, and is shown to be the case for purified cerebral histone kinase by the experiment illustrated in Fig. 2. Noradrenaline and histamine also increased cyclic AMP-independent histone kinase activity in mitochondrial and supernatant fractions respectively. Activation of this soluble enzyme may account, at least in part, for the stimulation of phosphate incorporation observed with these agents by Williams & Rodnight (1976). The present results support previous observations that histamine is more effective than noradrenaline as a stimulant of cyclic AMP formation in guinea-pig cortical slices (Shimizu *et al.*, 1969) and that correlation between activation of histone kinase and increased binding of endogenous cyclic AMP is more significant in the synaptosomal fraction, where the majority of the cyclic AMP is in a protein-bound form (Newman & McIlwain, 1978) than it is in other fractions.

Both adenosine and 2-chloroadenosine were without effect on histone kinase activity, although they both increased the bound cyclic AMP concentrations as shown by the decrease in binding capacity. This is in agreement with the results of Reddington *et al.* (1973), who obtained no effect of adenosine on basal phosphorylation nor on the increase in incorporation observed on electrical stimulation. These authors also found that adenosine inhibited the increase in phosphorylation produced by $0.1\ \text{mM}$ -histamine, and an inhibitory effect of adenosine was shown also on the phosphorylating activity of preparations of histone kinase *in vitro* (Miyamoto *et al.*, 1969) and for the endogenous protein kinase of ox synaptosomal membranes (Weller & Rodnight, 1973). The stimulating effect of adenosine deaminase on histone kinase activity in the nuclear fraction observed in the present work, which was most probably due to increased cyclic AMP-independent activity (Table 2), could be taken as evidence for an inhibitory action of endogenous adenosine, which then would be acting at a site different from that stimulating cyclic AMP formation (see also van Calker *et al.*, 1979). The existence of more than one binding site

for adenosine in subcellular preparations made from cerebral tissues has recently been demonstrated (Newman *et al.*, 1980, 1981); and it can be postulated that, whereas endogenous adenosine may react with any of these sites, externally added adenosine interacts only with the high-affinity receptor coupled to adenylate cyclase, which in this case is not linked to a phosphorylation reaction. Alternatively, added adenosine could be taken up into the cell, where it might have an inhibitory effect on the activity of the free catalytic subunit of histone kinase, as suggested by Severin *et al.* (1978).

Green & Noland (1979) have shown activation of cyclic AMP-dependent histone kinase and of endogenous protein phosphorylation by adenosine in C-1300 mouse neuroblastoma cells. The criteria of activation by salt and position of elution from a DEAE-cellulose ion-exchange column, however, showed that the supernatant histone kinase studied in the present work, and by inference the soluble enzyme in other fractions, was a type II enzyme, as found in other species by Corbin *et al.* (1975) and by Malkinson *et al.* (1978), whereas the same criteria applied to the work of Green & Noland (1979) show that they were dealing with a type I enzyme. This difference may explain the different behaviour to adenosine shown by the two preparations, even though both were of neural origin.

We are indebted to the Medical Research Council for support for these investigations.

References

- Corbin, J. D., Soderling, T. R. & Park, C. R. (1973) *J. Biol. Chem.* **248**, 1813–1821
- Corbin, J. D., Keely, S. L. & Park, C. R. (1975) *J. Biol. Chem.* **250**, 218–225
- Do Khac, L., Harbon, S. & Glauser, H. (1973) *Eur. J. Biochem.* **40**, 177–185
- Green, R. D. & Noland, T. A. (1979) *J. Supramol. Struct.* **10**, 125–135
- Greengard, P. (1976) *Nature (London)* **260**, 101–108
- Huang, M., Gruenstein, E. & Daly, J. W. (1973) *Biochim. Biophys. Acta* **329**, 147–151
- Kuo, J.-K. (1972) in *Methods in Cyclic Nucleotide Research* (Chasin, M., ed.), pp. 200–220, Dekker, New York
- Malkinson, A. M., Gharrett, A. J. & Hogy, L. (1978) *Biochem. J.* **175**, 367–375
- Marchbanks, R. M. (1975) in *Practical Neurochemistry* (McIlwain, H., ed.), pp. 208–242, Churchill Livingstone, London
- McIlwain, H. & Snyder, S. H. (1970) *J. Neurochem.* **17**, 521–530
- Miyamoto, E., Kuo, J. F. & Greengard, P. (1969) *J. Biol. Chem.* **244**, 6395–6402
- Newman, M. E. & McIlwain, H. (1978) *Biochem. J.* **170**, 73–79
- Newman, M. E., de Lucia, R., Patel, J. & McIlwain, H. (1980) *Biochem. Soc. Trans.* **8**, 141–142
- Newman, M. E., Patel, J. & McIlwain, H. (1981) *Biochem. J.* **194**, 611–620
- Pull, I. & McIlwain, H. (1972) *Biochem. J.* **130**, 975–981
- Reddington, M., Rodnight, R. & Williams, M. (1973) *Biochem. J.* **132**, 475–482
- Rodnight, R. (1979) *Int. Rev. Biochem.* **26**, 1–80
- Sattin, A. & Rall, T. W. (1970) *Mol. Pharmacol.* **6**, 13–23
- Severin, E. S., Sashchenko, L. P., Kochetkov, S. N. & Kurochkin, S. N. (1978) *Adv. Cyclic Nucleotide Res.* **9**, 171–184
- Shimizu, H., Daly, J. W. & Creveling, C. R. (1969) *J. Neurochem.* **16**, 1609–1619
- Taylor, K. M. & Snyder, S. H. (1973) *J. Neurochem.* **21**, 1215–1223
- van Calker, D., Miller, M. & Hamprecht, B. (1979) *J. Neurochem.* **33**, 999–1005
- Walsh, D. A., Ashby, C. D., Gonzalez, C., Calkins, D., Fisher, E. H. & Krebs, E. G. (1971) *J. Biol. Chem.* **246**, 1977–1985
- Weller, M. & Rodnight, R. (1973) *Biochem. J.* **132**, 483–492
- Williams, M. & Rodnight, R. (1976) *Biochem. J.* **154**, 163–170