Biochemical Pharmacology, Vol. 23, pp. 835-843. Pergamon Press, 1974. Printed in Great Britain.

ABSENCE OF AN EFFECT OF HISTAMINE, NORADRENALINE AND DEPOLARIZING AGENTS ON THE LEVELS OF ADENOSINE 3', 5'-MONOPHOSPHATE IN NERVE ENDINGS ISOLATED FROM CEREBRAL CORTEX

JACQUELINE S. DE BELLEROCHE, INDRAJIT DAS and H. F. BRADFORD

Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AY, England

(*Received* 11 *July* 1973; *accepted* 13 *August* 1973)

Abstract—Adenosine 3',5'-monophosphate levels were measured in beds of incubated synaptosomes from rat cerebral cortex and found to be 15–17 pmoles/mg protein (insoluble protein after ethanol extraction) or 9–13 pmoles/mg protein (TCA precipitated). Periods of incubation up to 60 min did not cause a change in these levels. Depolarizing agents such as electrical pulses and elevated medium K^+ and a physiologically active compound, noradrenaline, which did produce large increases in the adenosine 3',5'-monophosphate of rat cerebral cortex slices did not alter the adenosine 3',5'-monophosphate levels above those of control synaptosome beds. Histamine, which was also without effect on adenosine 3',5'-monophosphate levels, caused a small but significant increase in the glutamate, GABA and aspartate released to the medium, which was qualitatively similar to the larger effect produced by electrical and K^+ stimulation. The small amount of adenosine 3',5'-monophosphate lost to the medium during incubation (< 10 per cent) was not altered in response to any of the agents used. Phosphodiesterase activity was also measured but showed no change following application of electrical pulses, or in the presence of elevated medium K^+ (56 mM). High concentrations of theophylline in the medium were also without significant effect on synaptosomal adenosine 3',5'-monophosphate levels. It was verified that noradrenaline and not histamine produces a significant increase in the adenosine 3',5'-monophosphate of rat cerebral cortex slices. A postsynaptic site for the action of adenosine 3',5'-monophosphate is proposed.

BRAIN contains very high activities of enzymes associated with adenosine 3',5'-monophosphate synthesis, namely, adenylate cyclase,[1] its degradive enzyme, phosphodiesterase[2] and a phosphoprotein kinase which is stimulated by adenosine 3',5'-monophosphate.[3,4] These three enzymes or enzyme systems have further been shown to be especially concentrated in nerve endings isolated from the brain[5–9] and the phosphoprotein kinase has been further localized to synaptic membrane fractions and synaptic vesicles.[7] These observations indicate that adenosine 3',5'-monophosphate may play a part in neurotransmission itself or in processes accompanying neurotransmission. Considerable increases in the levels of adenosine 3',5'-monophosphate are certainly shown in response to depolarizing agents, histamine and catecholamines in mammalian brain slices[10–13] and it appeared possible that the effect was at the nerve ending. In order to examine this possibility, adenosine 3',5'-monophosphate was measured in beds of isolated nerve terminals. Incubation in this fashion allows optimal performance of isolated nerve endings in terms of response and recovery following exposure to depolarizing stimuli. Thus, putative transmitters such as

physiologically active amino acids and acetyl choline are released under these conditions by calcium-dependent processes,[14, 15] suggesting that the isolated nerve endings are at least partially working presynapses, in which one could reasonably expect any system involving adenosine 3',5'-monophosphate to maintain part of its activity.

MATERIALS AND METHODS

Adult female Sprague–Dawley rats (200–250 g) were used in all these studies.

Preparation of synaptosome beds and cerebral cortex slices. The cerebral cortex was removed from 15 rats and used to prepare eight synaptosome beds. Synaptosomes were prepared by the method of Gray and Whittaker[16] with modifications as described by Bradford[17] and the use of shorter centrifugation times as follows: pelleting of the crude mitochondrial pellet at 17,500 g for 20 min and centrifugation of the discontinuous sucrose gradient for 1 h at 75,000 *g*. Pellets from the synaptosome fraction were gently resuspended in Krebs–bicarbonate medium of composition: (mM); NaCl, 124; KCl, 5; KH_2PO_4, 1·2; $MgSO_4$, 1·3; $CaCl_2$, 0·75; $NaHCO_3$, 26 mM; pH 7·5 containing 10 mM glucose. Portions of the suspension were sedimented onto rectangles of nylon gauze (1·7 × 2·4 cm), and identical pieces of gauze were placed on top of the deposit to form synaptosome beds. The full details of this procedure are described elsewhere.[14] The synaptosome bed was manipulated by gripping the bare edge of the nylon gauze with forceps, the synaptosome deposit remaining intact between the gauzes.

Cerebral cortex slices were cut from the outer surface of the whole cerebral cortex using a strip of razor blade and a glass guide recessed to give slices 350–400 μm in thickness.[18]

Incubation of synaptosome beds and cortex slices. Both synaptosome beds and cortex slices were held between the jaws of Quick Transfer Holders as described for slices by McIlwain and Rodnight.[18] The Holder was then dipped into medium, contained in a small beaker (25 ml), thereby immersing the slice or bed in the medium. Incubation was carried out at 37° in Krebs–bicarbonate medium (5 ml) of composition described above. The medium was gassed with 95% O_2/5% CO_2 and additions of compounds to the medium were made at the point of gassing to ensure rapid mixing. Electrical stimulation was by application of square wave pulses of 10 V, 0·4 msec duration and alternating in polarity at the frequency of 100/sec (average continuous current 35 mA) to the electrodes of the Quick Transfer Holder which are held on either side of the tissue sample.[14]

Extraction. Adenosine 3',5'-monophosphate was extracted by either ethanol or TCA extraction as indicated in the legends. By the former method, at the end of incubation, the synaptosome beds were placed in 80% ethanol (5 ml/7 mg synaptosome bed), mixed, left in ice for 10 min and centrifuged at 9000 *g* for 15 min. The resulting pellet was further extracted with 60% ethanol (5 ml), recentrifuged and the combined supernatants were evaporated to dryness. By the second method, synaptosome beds were placed in TCA (3 ml), mixed, and centrifuged at 9000 *g* for 15 min. The pellet was retained for protein estimation and the supernatant was extracted three times with three volumes of water-saturated ether to remove TCA into the ether extract. The aqueous fraction was dried. Cerebral cortex slices were extracted with 5% TCA with homogenization, and then treated as above.

At the end of incubation, the incubation medium was freeze dried. Where amino acid analysis was carried out, a small portion of the medium (1 ml) was first removed and this was placed in 1·0 M PCA (3 ml).

Adenosine 3′,5′-monophosphate determination. The dry solid was redissolved in 50 mM Tris–HCl buffer, pH 7·4 (containing 8 mM theophylline and 6 mM 2-mercaptoethanol). Adenosine 3′5′-monophosphate was then estimated by the method of Brown *et al.*[19] Each assay was performed at two dilutions and accompanied by standard control. ^{3}(H) adenosine 3′,5′-monophosphate was obtained from the Radiochemical Centre, Amersham, Bucks, U.K.

Adenosine, 3′,5′-monophosphate phosphodiesterase assay. The enzyme from incubated synaptosome beds was extracted with 0·25 M sucrose, 0·04 M Tris–HCl, pH 7·5 (7 mg of synaptosomal protein/ml of buffer) and then centrifuged at 1000 *g* for 20 min at 4°. Adenosine 3′,5′-monophosphate phosphodiesterase was assayed in the supernatant by the method described by Butcher and Sutherland[2] with some modifications.[20] We used alkaline phosphatase to hydrolyze 5′ AMP instead of crotalus atrox venom. The assay mixture contained 40 mM Tris–HCl, 2 mM $MgSO_4$ and 1 mM adenosine 3′,5′-monophosphate at pH 7·5. The reaction was terminated by the addition of cold 20% TCA containing 1% activated charcoal (1 ml). One ml of this solution was used for phosphorus determination as described by Dryer *et al.*[21] Adenosine 3′,5′-monophosphate and alkaline phosphatase were purchased from Boehringer Co., U.K.

Other analytical methods. Amino acids were analyzed by automated amino acid analysis as described by Bradford and Thomas.[22] Protein was estimated by the method of Lowry *et al.*[23]

RESULTS

The adenosine 3′,5′-monophosphate content of incubated synaptosome beds and the effect of depolarizing agents. When synaptosome beds were incubated in Krebs-bicarbonate medium and stimulated by elevated medium K^+ or electrical pulses, there was no change in the adenosine 3′,5′-monophosphate content of the synaptosome beds compared to the unstimulated beds (Table 1). The loss of adenosine 3′,5′-monophosphate to the medium during incubation was small (8–10 per cent of the total) and similarly was found to show no change with both types of stimulation (Table 1). Incubation of synaptosome beds in medium containing theophylline (10 mM), as an inhibitor of phosphodiesterase (the enzyme catabolizing adenosine 3′,5′-monophosphate) also did not greatly alter the synaptosomal levels of adenosine 3′,5′-monophosphate (Table 1). Control levels (35 min incubation) of adenosine 3′,5′-monophosphate in synaptosome beds showed a 9·3 per cent drop in the presence of theophylline.

The effect of time of electrical stimulation on synaptosomal levels of adenosine 3′,5′-monophosphate. The conditions chosen for stimulation i.e. high medium K^+ or application of electrical pulses for a period of 10 min were those found to give a significant or optimal (in the case of electrical stimulation) release of physiologically active compounds (acetyl choline, GABA, glutamic acid and aspartic acid) from synaptosome beds.[14, 15] However, in order to detect either short term or delayed changes, two other periods of electrical stimulation were used. Electrical pulses applied for either 5 or 30 min (35 or 60 min total incubation respectively as there was an initial 30

TABLE 1. ADENOSINE 3′,5′-MONOPHOSPHATE LEVELS AND PHOSPHODIESTERASE ACTIVITY IN INCUBATED SYNAPTOSOME BEDS AND THE EFFECT OF POTASSIUM AND ELECTRICAL STIMULATION

	Adenosine 3′,5′-monophosphate (pmoles/mg synaptosome bed protein)		Phosphodiesterase activity (nmoles adenosine 3′,5′-monophosphate catabolized/mg protein/min)
	Synaptosome bed	Released to the medium	Synaptosome bed
40 min incubation			
Control	16·42 ± 1·12(6)	1·6 ± 0·1(4)	3·98 ± 0·15(6)
K^+ stimulation (10 min)	17·57 ± 0·66(5)	1·4 ± 0·05(4)	4·03 ± 0·17(5)
Electrical stimulation (10 min)	17·20 ± 1·39(5)		3·62 ± 0·36(5)
35 min incubation			
Control	17·13 ± 0·53(5)	—	—
Control with theophylline 10 mM	15·52 ± 0·89(5)	—	—
Electrical stimulation (5 min)	16·13 ± 1·01(5)	—	—
Electrical stimulation with theophylline 10 mM (5 min)	16·12 ± 0·80(5)	—	—
60 min incubation			
Control	15·60 ± 1·48(5)	—	—
Electrical stimulation (30 min)	15·37 ± 1·77(5)	—	—

Synaptosome beds were incubated in Krebs–bicarbonate medium containing 10 mM glucose at 37° for the incubation periods indicated above. Electrical or K^+ stimulation was commenced after an initial incubation period of 30 min. Electrical stimulation of the synaptosome bed was by the application of electrical pulses till the end of incubation and K^+ stimulation was brought about by raising the medium K^+ from 6 to 56 mM. Values are means ± S.E. of the means for the no. of experiments in brackets. Ethanol (80/60%) was used for extraction of adenosine 3′,5′-monophosphate.

min period of incubation in all cases) did not cause a significant increase in the adenosine 3′,5′-monophosphate levels above the control values (Table 1).

Phosphodiesterase activity. The appearance of a change in adenosine 3′,5′-monophosphate concentrations may well have been prevented by an increased activity of adenosine 3′,5′-monophosphate phosphodiesterase due to the same stimuli. For this reason the activity of this enzyme was measured under the same conditions as above, and was found to show no change in response to electrical pulses or raised medium K^+ (Table 1).

The effect of noradrenaline and histamine on synaptosomal adenosine 3′,5′-monophosphate. Incubation of synaptosome beds in concentrations of noradrenaline and histamine which produce large increases in the adenosine 3′,5′-monophosphate of cerebral cortex slices did not have any measurable effect on the adenosine 3′,5′-monophosphate of the synaptosome bed or the small portion released to the medium (Table 2).

TABLE 2. THE EFFECT OF NORADRENALINE AND HISTAMINE ON THE LEVELS OF ADENOSINE 3',5'-MONOPHOSPHATE IN INCUBATED CEREBRAL CORTEX SLICES AND SYNAPTOSOME BEDS

	Adenosine 3',5'-monophosphate			
	pmoles/mg bed protein		pmoles/mg wet wt of cortex slice	
	Synaptosome bed	Released to the medium	Cerebral cortex slice	Released to the medium
Control	11·16 ± 1·25(4)	0·80 ± 0·06(4)	4·49 ± 0·30(13)	0·74 ± 0·17(13)
Control with phenylalanine (0·05 M)	11·32 ± 0·28(4)	0·72 ± 0·15(4)	—	—
Noradrenaline present (0·5 mM)	12·87 ± 0·59(8)	0·81 ± 0·09(8)	10·98 ± 1·11(8)*	0·68 ± 0·06(8)
Histamine present (0·1 mM)	—	—	3·93 ± 0·36(5)	0·78 ± 0·22(5)
Theophylline present in the medium (0·5 mM)				
Control	9·00 ± 0·81(7)	0·51 ± 0·28(7)	2·22 ± 0·45(5)	1·05 ± 0·26(5)
Histamine present (0·1 mM)	9·60 ± 0·87(7)	0·50 ± 0·05(7)	2·27 ± 0·32(5)	1·04 ± 0·24(5)

* Significantly greater than the control ($P < 0·01$).

Synaptosome beds or cerebral cortex slices were incubated in Krebs–bicarbonate medium containing 10 mM glucose at 37° for 40 min. The medium contained theophylline (0·5 mM) where indicated. Additions of histamine, phenylalanine and noradrenaline were made after 30 min incubation to give final concentrations in the medium of 0·1, 0·5 and 0·5 mM respectively and at the same time equivalent volumes of medium were added to the controls. Extraction of synaptosome beds was carried out using 5% TCA. Values of adenosine 3',5'-monophosphate are means ± S.E. of the means for the number of experiments in brackets.

Theophylline itself (0·5 mM) was shown to produce a small decrease (19·3 per cent) in the levels of adenosine 3′,5′-monophosphate in control samples. Phenylalanine (0·5 mM), dissolved in dilute acid, as for noradrenaline, was added to the media of some control synaptosome beds to provide a control consisting of a structurally related but physiologically inactive compound, and this also produced no change. The values referred to in this paragraph, were obtained from TCA extracts of the synaptosome beds and were about 40 per cent less than the values obtained from 80/60% ethanol extracts (Table 1). The difference is due to the loss of ethanol soluble protein in the latter case reducing the protein content of the bed and thus artificially elevating the values expressed on a protein basis. Adenosine 3′,5′-monophosphate values thus appear smaller when the TCA method of extraction is used.

Although all three agents were without effect on synaptosomal levels of adenosine 3′,5′-monophosphate, histamine did however, produce a significant release of the physiologically active amino acids, glutamate, GABA and aspartate (Table 3). This pattern qualitatively resembles the release, previously reported to occur, from cerebral cortex synaptosome beds in response to elevated medium K^+ (56 mM) and electrical pulses[14] but in quantitative terms it was considerably smaller (about 50 per cent). On the other hand noradrenaline (0·5 mM) only caused a significant increase in the amino acid glycine, all other amino acids being unaffected. The effects of glycine appear to be in contrast to those of the other agents studied and this is being further investigated. The presence of phenylalanine did not lead to any significant increases in the release of physiologically active amino acids from synaptosomes. However, the increases in alanine and glutamine and decrease in aspartate occurring under these conditions may be a result of interference on the part of phenylalanine with the uptake systems, especially those of structurally similar amino acids.

The effect of histamine and noradrenaline on the adenosine 3′,5′-monophosphate of incubated cerebral cortex slices. The effects of noradrenaline and histamine on brain slice adenosine 3′,5′-monophosphate are fairly well established and were repeated in the present studies in order to check the method of extraction and assay. Incubation of cerebral cortex slices for 10 min in the presence of noradrenaline (0·5 mM) led to a significant increase (144·5 per cent) in the adenosine 3′,5′-monophosphate of the slice (Table 3) which was at the lower end of the range (120–2000 per cent increase) previously reported for rat brain slices.[24–27]

The large differences in the degree of increase in adenosine 3′,5′-monophosphate with noradrenaline are partly a consequence of the method used for measurement. The values at the lower end of the range are increases in endogenous adenosine 3′,5′-monophosphate, while the higher values are increases in the amount of radioactively labelled adenosine 3′,5′-monophosphate formed from a labelled precursor such as adenine [^{14}C] during a short period of incubation. In the latter case the pool of adenosine 3′,5′-monophosphate formed prior to incubation will not become labelled to any great extent and the unstimulated sample will have a low value of radioactively labelled adenosine 3′,5′-monophosphate associated with it rather than the absolute level measured by the former method.

Histamine (0·1 mM) was found to be without effect on rat cortex slices as reported by other workers (Table 2 and Ref. 24). The presence of theophylline (0.5 mM) in the incubation medium of cerebral cortex slices produced a significant decrease in the

Table 3. THE EFFECT OF HISTAMINE AND NORADRENALINE ON AMINO ACID RELEASE FROM INCUBATED SYNAPTOSOME BEDS

	nmoles of amino acid released/100 mg protein				
	Incubation in medium containing theophylline (0·5 mM)		No Theophylline		
	Control	Histamine (0·1 mM)	Control	Control with phenylalanine (0·5 mM)	Noradrenaline (0·5 mM)
Aspartate	213·3 ± 22·8	300·8 ± 34·6*	165·4 ± 10·6	121·2 ± 11·0§	163·7 ± 39·8
Glutamine	534·8 ± 44·1	557·3 ± 21·3	419·5 ± 18·7	605·3 ± 66·8*	398·1 ± 33·2
Serine	724·2 ± 64·2	698·9 ± 24·9	571·8 ± 24·6	674·0 ± 93·0	512·7 ± 22·2
Glutamate	376·1 ± 41·5	524·4 ± 55·4*	227·3 ± 16·2	227·5 ± 22·1	232·6 ± 52·8
Glycine	479·1 ± 29·6	495·3 ± 17·7	367·1 ± 19·9	455·5 ± 22·8	518·8 ± 35·9‡
Alanine	495·2 ± 46·4	523·7 ± 30·6	406·3 ± 9·0	755·5 ± 61·8‡	486·8 ± 46·5
GABA	79·5 ± 6·4	129·7 ± 16·6†	52·2 ± 3·9	52·0 ± 14·9	44·6 ± 28·5
No. of experiments	7	7	4	4	8

Synaptosome beds were incubated in Krebs–bicarbonate medium for 40 min at 37°. The medium contained theophylline (0·5 mM), where indicated. Phenylalanine, histamine and noradrenaline were added to the incubation medium after 30 min incubation to give medium concentrations of 0·5, 0·1 and 0·5 mM respectively. Values are means ± S.E. of the means.

* Significantly greater than control $0{\cdot}1 > P > 0{\cdot}05$.

† Significantly greater than control $0{\cdot}05 > P > 0{\cdot}02$.

‡ Significantly greater than control $0{\cdot}02 > P > 0{\cdot}01$.

§ Significantly less than control $0{\cdot}1 > P > 0{\cdot}05$.

levels of slice adenosine 3′,5′-monophosphate as has already been reported by Kakiuchi *et al.*[12] but the medium levels were slightly increased.

DISCUSSION

Despite the considerable activity of adenosine 3′,5′-monophosphate enzymes in nerve ending fractions,[5–9] no response was shown to electrical pulses, raised K^+ or biogenic amines under the conditions of incubation of synaptosome beds used in the present experiments. The significant changes in adenosine 3′,5′-monophosphate caused by these agents in brain slices must indicate that another component not present in nerve endings is responsible for translating the effect of depolarization or amine stimulation and hence triggering off increased adenosine 3′,5′-monophosphate production.

A great deal of evidence has now accrued from this and other laboratories demonstrating that synaptosomes possess many of the properties expected of intact, metabolically active, cytoplasmic bodies, and display some of the basic properties shown by the presynapse *in situ*, namely, released putative transmitters by calcium-dependent processes in response to depolarizing agents,[14, 15, 28, 29] and release of hypophysiotrophic hormones by similar mechanisms.[30] Whether the incubated synaptosome can be confidently regarded as an isolated working presynapse clearly remains to be established. However it does provide a preparation in which any presynaptic process involving cyclic AMP could be expected to show activity. Thus, considerable ATP and phosphocreatine production by synaptosomes has been demonstrated[14, 17] and therefore any effect due to limited availability of ATP is likely to be minimal. In spite of these apparently favourable circumstances, no change in cyclic AMP levels was detected following treatment with the various agents, some of which were showing marked effects in releasing putative transmitters. Therefore with the limitations drawn out above, it appears that the increased cyclic AMP levels observed to occur in cortex slices, are not localized in the pre-synapse, though actions of this compound in the presynapse not involving a changed concentration might still be occurring.

It seems therefore, that changes in cyclic AMP may be occurring postsynaptically, even involving the whole postsynaptic body. This conclusion is well supported by biochemical and pharmacological studies carried out by Greengard and his coworkers using the superior cervical ganglion. By this technique, they have shown that cyclic AMP increases in the ganglion itself in response to pre-ganglionic stimulation[31] or application of dopamine, and probably occurs due to stimulation of the dopaminergic interneurone.[32] A post synaptic site of stimulation of cyclic AMP is also strongly implied from the demonstration of Chasin *et al.*[33] that agents which block α and β receptors also inhibit cyclic AMP rises in guinea pig cerebellum slices in response to histamine and noradrenaline. Inactivation by phosphodiesterase could also occur in the immediate vicinity of the postsynaptic membrane in which its activity has been demonstrated.[34]

The post synaptic thickening is, in fact, seen on many but not all synaptosomes,[35] but since the sections are only 50–60 nm thick whilst synaptosomes are 1 μm in diameter; this could be due to the planes of section simply missing the thickening. Furthermore, De Robertis *et al.*[36] obtained an impressively large yield of postsynap-

tic thickenings when other structures were dissolved away with detergents, suggesting that synaptosomes do, in fact, carry a postsynaptic thickening.

The presence of a postsynaptic membrane in synaptosome preparations could therefore provide one explanation for the presence of high concentrations of enzymes associated with cyclic AMP, but in the absence of more of the post synaptic cell, activation of these enzymes is presumably not possible.

Acknowledgements—This work was carried out with financial support in the form of an M.R.C. project grant for which we are most obliged. We are also grateful to Professor Sir Ernst Chain, F.R.S., for encouragement. The technical assistance of H. Wille and N. Freund is gratefully acknowledged.

REFERENCES

1. E. W. Sutherland, T. W. Rall and T. Minon *J. biol. Chem.* **237,** 1220 (1962).
2. R. W. Butcher and E. W. Sutherland, *J. biol. Chem* **237,** 1244 (1962).
3. E. Miyamoto, J. F. Kuo and P. Greengard, *J. biol. Chem.* **244,** 6395 (1969).
4. M. Weller and R. Rodnight, *Nature, Lond.* **225,** 187 (1970).
5. E. de Roberts, G. Rodriguez de Lores Arnaiz, M. Alberici, R. W. Butcher and E. W. Sutherland, *J. biol. Chem.* **242,** 3487 (1967).
6. B. Weiss and E. Costa, *Biochem. Pharmac.* **17,** 2107 (1968).
7. H. Maeno, E. M. Johnson and P. Greengard, *J. biol. Chem.* **246,** 134 (1971).
8. S. Gaballah and C. Popoff, *J. Neurochem.* **18,** 1795 (1971).
9. S. Gaballah and C. Popoff, *Brain Res.* **25,** 220 (1971).
10. S. Kakiuchi and T. W. Rall, *Mol. Pharmac.* **4,** 367 (1968).
11. S. Kakiuchi and T. W. Rall, *Mol. Pharmac.* **4,** 379 (1968).
12. S. Kakiuchi, T. W. Rall and H. McIlwain, *J. Neurochem.* **16,** 485 (1969).
13. H. Shimizu, J. W. Daly and C. R. Creveling, *J. Neurochem.* **16,** 1609 (1969).
14. J. S. de Belleroche and H. F. Bradford, *J. Neurochem.* **19,** 585 (1972).
15. J. S. de Belleroche and H. F. Bradford, *J. Neurochem.* **19,** 1817 (1972).
16. E. G. Gray and V. P. Whittaker, *J. Anat., Lond.* **96,** 79 (1962).
17. H. F. Bradford, *J. Neurochem.* **16,** 675 (1969).
18. H. McIlwain and R. Rodnight, in *Practical Neurochemistry.* Churchill, London (1962).
19. B. L. Brown, J. D. M. Albano, R. P. Ekins, A. M. Sgherzi and W. Tampion, *Biochem. J.* **121,** 561 (1971).
20. I. Das, *Horm. metab. Res.* **5,** 330 (1973).
21. R. L. Dryer, A. R. Tammes and J. I. Routh, *J. biol. Chem.* **255,** 177 (1957).
22. H. F. Bradford and A. J. Thomas, *J. Neurochem.* **16,** 1495 (1969).
23. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
24. J. Forn and G. Krishna, *Fedn Proc.* **29,** 480, Abst. No. 1326 (1970)
25. E. C. Palmer, G. A. Robinson, A. A. Manian and F. Sulser, *Psychopharmacologia (Berl)* **23,** 201 (1972).
26. G. Krishna, J. Forn, K. Voigt, M. Paul and G. L. Giessa, *Advan Biochem. Pharmac.* **3,** 155 (1970).
27. H. Shimizu, C. R. Greveling and J. Daly, *Proc. natn. Acad. Sci. U.S.A.*, **65,** 1033 (1970).
28. M. P. Blaustein, E. M. Johnson, jr. and P. Needleman, *Proc. natn. Acad. Sci. U.S.A.* **69,** 2237 (1972).
29. R. H. Osborne, H. F. Bradford and D. Jones, *J. Neurochem.* **21,** 407 (1973).
30. J. A. Edwardson, G. W. Bennett and H. F. Bradford, *Nature, Lond.* **240,** 554 (1972).
31. D. A. McAfee, M. Schorderet and P. Greengard, *Science, N.Y.* **171,** 1156 (1971).
32. J. W. Kebabian and P. Greengard, *Science, N.Y.* **174,** 1346 (1971).
33. M. Chasin, I. Rivkin, F. Mamrak, S. G. Samaniego and S. M. Hess, *J. biol. Chem.* **246,** 3037 (1971).
34. N. T. Florendo, R. J. Barrnett and P. Greengard, *Science, N.Y.* **173,** 745 (1971).
35. D. G. Jones and H. F. Bradford, *Tissue and Cell* **3,** 177 (1971).
36. E. de Robertis, J. M. Azcurra and S. Fisza, *Brain Res.* **5,** 45 (1967).