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COMMENTARY

THE POLYAMINES IN THE CENTRAL NERVOUS SYSTEM

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The polyamines putrescine, spermidine and spermine owe their trivial names to an association with putrefying matter or seminal fluid, yet they are ubiquitous in body tissues. Spermidine and spermine are to be found in high concentrations in nervous tissue. Indeed, a basic compound named neuridine which had been isolated from brain tissue in 1885 was subsequently established to be identical with spermine. The term polyamine is perhaps something of a misnomer since it suggests a high molecular weight compound with multiple amine residues whereas putrescine is a simple diamine: $H_2N(CH_2)_4$-NH_2 and spermidine and spermine are low molecular weight bases with three $H_2N(CH_2)_3NH(CH_2)_4NH_2$ and four $H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$ basic centres, respectively. However the metabolic interrelations of these compounds make it desirable that they should be classified together.

The biological role of polyamines is the subject of several reviews and monographs [1–6] but these are mainly concerned with peripheral tissues or bacteria since most research has hitherto concentrated in those areas. It is now opportune to review the progress that has been made in the relatively neglected area of the study of central nervous functions of polyamines.

SYNTHESIS, DISTRIBUTION AND METABOLISM

It is generally accepted that the main pathway for polyamine synthesis is the same in the central nervous system as in peripheral tissues, though the characteristics of the enzymes concerned may vary from tissue to tissue. In essence, putrescine is synthesised from ornithine by ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) and converted to spermidine by a specific synthase (5′-deoxyadenosyl-(5′), 3-aminopropyl-(1), methylsulphonium salt: putrescine 3-aminopropyl transferase, EC 2.5.1.16) which utilises decarboxylated *S*-adenosyl methionine as a propylamine donor. A spermine synthase (5′-deoxyadenosyl-(5′), 3-aminopropyl-(1), methylsulphonium salt: spermidine 3-aminopropyl transferase) converts spermidine to spermine using the same donor. All the enzymes involved in polyamine biosynthesis are soluble [7].

Ornithine decarboxylase has a half life of only 10–20 min which is probably related to rapid enzyme degradation [7] but which could also be explained by dissociation of the loosely bound pyridoxal cofactor [8]. The rat brain enzyme has a higher affinity for ornithine than the liver enzyme and is only weakly inhibited by putrescine [9]. Changes in ornithine decarboxylase activity take place during early brain development, activity being generally increased in rat brain from the 14th day after conception to the 12th post natal day but showing regional variation. The greatest activity generally parallels periods of cell proliferation. The clear post-partum peak of activity which occurs 4 hr after birth is prevented by administration of cycloheximide suggesting that *de novo* enzyme synthesis is the prime determinant of activity [10] though more recently a cycloheximide sensitive inhibitor protein has been detected in thyroid tissue [11]. In adrenal medulla there is evidence that ornithine decarboxylase activity is regulated by c-AMP and experiments with glioma and neuroblastoma cells have shown that agents such as noradrenaline and prostaglandin E which increase cellular c AMP content also increase ornithine decarboxylase activity [12]. Brain putrescine content is increased by the administration of L-DOPA [13].

There is now evidence to indicate that ornithine decarboxylase activity is a sensitive index of brain maturation in that the pattern of change in activity in developing brain is accelerated and compressed by thyroxine which accelerates maturation, and extended and depressed by cortisol which slows maturation [14]. Brain ornithine decarboxylase activity is also stimulated by growth hormone, prolactin, human placental lactogen [15] and nerve growth factor [16] and by insulin. The effect of insulin is unrelated to hypoglycaemia but may be exerted through an adenylate cyclase since it is suppressed by lithium [17]. Administration of opiates to newborn rats or to their mother delayed changes in ornithine decarboxylase activity [18, 19] whereas ethanol administration produced less dramatic effects which were dependent on the duration of exposure to ethanol and the time of withdrawal [20, 21]. More recent experiments indicate that removal of rat pups from their mother for as little as one hour produces a significant decline in ornithine decarboxylase activity. Since this decline was also present in pups allowed access to an anaesthetised mother which was able to support suckling, it was concluded that the decline was related to deprivation of normal 'mothering' behaviour [22, 23].

In normal adult brain ornithine decarboxylase activity is low but it increases after ischaemia [24] and after convulsions produced by electrical stimulation [25]. Electrical stimulation of snail brain results in an increase in its putrescine content [26].

Brain *S*-adenosyl methionine decarboxylase (*S*-adenosyl-L-methionine carboxy-lyase EC 4.1.1.50) is strongly putrescine dependent but probably not

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pyridoxal dependent. Activity in rat brain remains low until after the 10th post natal day and subsequently increases [27]. In the monkey, adult values are reached after one month and thereafter remain constant [28]. Increased *S*-adenosyl methionine decarboxylase activity, like that of ornithine decarboxylase, has been reported to follow electrical stimulation or ischaemia [24, 25] but the response occurs later than the ornithine decarboxylase response and may in part be a response to increased putrescine concentration arising from ornithine decarboxylase activity. This for example might explain the first component of the biphasic response seen after electrical stimulation. Like ornithine decarboxylase this enzyme has a short half life (20–60 min) but its strong dependence on putrescine suggests that it might not be rate limiting. This suggestion is supported by the observation that, at 18 days, the brain spermidine and spermine content of dilute lethal mice is normal whereas the brain *S*-adenosyl methionine decarboxylase activity is only 20 per cent of normal [29]. In addition there is no correlation between brain spermidine and spermine content and *S*-adenosyl methionine decarboxylase activity during early brain development [30]. However, methylglyoxal bis (guanyl hydrazone) which is a potent and fairly selective inhibitor of the enzyme [31] does inhibit spermidine synthesis in rat brain [32].

Spermidine synthase is a stable enzyme with a much greater activity than *S*-adenosyl methionine decarboxylase and a specific requirement for putrescine [33]. Spermine synthase is also stable but its activity is lower than that of spermidine synthase. It is strongly inhibited by putrescine and has a specific requirement for spermidine. The activity of this enzyme is probably rate limited by the availability of propylamine for which it has a high affinity. In brain, synthase activity is clearly separable from decarboxylase activity [33].

The polyamines have been detected in the brains of all species so far examined. During brain development the putrescine content directly reflects ornithine decarboxylase activity. As might be expected from the reduction in ornithine decarboxylase activity which takes place before adulthood, putrescine is present in the brains of most adult mammals and birds in a concentration of only a few nmoles/g which is only 2–3 per cent of the spermidine concentration. However, in fish brain the putrescine content exceeds that of spermidine or spermine [34]. The low concentration of putrescine in mammalian brain makes accurate quantitation difficult and estimates vary with different methodology. Perhaps the most acceptable estimates are those obtained by mass spectrometry of the dansyl derivative. These show a wide variation in the putrescine content of different brain regions with higher concentrations in the spinal cord, cerebellum, hypothalamus and cerebral cortex [35]. Values obtained by enzymatic assay, whose specificity is suspect, are higher and more uniform [36].

During early brain development in the rat there is a reduction in both spermidine and spermine concentration over the first three post natal weeks [30] but over the next 20 months spermine concentrations remain constant as do those of spermidine except in the hypothalamus, corpus striatum and pons-medulla where increases are reported [37]. Since brain weight increases rapidly over the first few months of life, polyamine synthesis must be maintained in order to sustain these near constant concentrations. In adult animals brain spermidine concentration shows wide regional variation. The highest concentrations, approaching 100 nmoles/g, are to be found in the brain stem and spinal cord and in regions composed mainly of white matter [35, 38] whereas amounts in grey matter are low. In general, the concentration of spermidine parallels the white matter content of the area though higher values than might be predicted on this basis have been reported in the colliculi [38, 39] and in the hypothalamus of the monkey [39].

The spermine content of the brain is in most species lower than that of spermidine [38, 40] a finding which concurs with the relative activity of the respective synthases. The distribution of spermine is much more uniform than that of spermidine but there is general agreement that levels in cerebellum are high and, in the rat, high concentrations are reported in the olfactory lobes [35, 38]. Some studies have reported high levels in the cerebral cortex [30, 41] but this is not a consistent finding.

Very little information is available concerning the degradation of spermidine and spermine. One reason for this is that the process is very slow. In rat brain spermidine formed from putrescine has a half-life of several days and degradation of spermine is not detectable over a two week period [42, 43]. It is usually assumed that polyamines are metabolised by an oxidative process since putreanine [44, 45] and spermic acid [46] are both present in brain. These are, respectively, the carboxylic acids which would be produced by oxidative deamination of the aminopropyl residue of spermidine or of both residues of spermine. The corresponding aldehydes would be unstable [47]. Oxidase activity has been demonstrated in chick embryo brain [48] and incorporation of label from spermidine into putreanine has been detected in rat brain [49] and in cultured mouse neuroblastoma cells [50] but the enzyme itself has not yet been isolated from brain. It is now becoming clear that interconversion of polyamines can also take place. Putrescine can be formed from spermidine [50] and the conversion of spermine to spermidine has been reported in rabbit brain [51] and in retina [52]. These same conversions can be carried out by the very active oxidase found in ruminant serum and result in the formation of the cytotoxic aldehyde acrolein [53] as a degradation product of the aminopropyl residue. Clearly, there is a requirement for further study of these pathways and for characterisation of the brain oxidative enzyme.

It should be mentioned that putrescine is also converted to gamma-aminobutyric acid [54–56].

RELATIONSHIPS WITH NUCLEIC ACIDS AND STRUCTURAL ELEMENTS OF BRAIN CELLS

Interactions between polyamines and nucleic acids were reported early in the history of polyamine research and have occupied the attention of many workers ever since. The initial observations of binding with and stabilisation of nucleic acids have been extended to reveal possible roles for polyamines in

nucleic acid and protein synthesis. Thus, in peripheral tissues or bacteria spermidine has been shown to be required for optimal activity of DNA replicase, DNA-dependent RNA polymerase and *t* RNA methyl transferase, to overcome inhibition of ribonuclease by Poly A or Poly G and to stabilise ribosomes. Inhibition of ornithine decarboxylase in hepatoma cells stopped cell proliferation and the incorporation of thymidine into DNA and addition of putrescine, spermidine or spermine restored cell replication. In chick embryo brain, spermidine and spermine accelerated the incorporation of formate into both DNA and RNA and led to the formation of polyribosome aggregates [48]. Intracisternal injection of putrescine into rats accelerates uridine incorporation into RNA [57], and spermidine and spermine increased valine incorporation into a microsomal fraction of rat cortex [58]. Many observations have confirmed that polyamine synthesis is accelerated in rapidly dividing tissues and it is now clear that blood polyamine levels can be used as markers of cancer and other proliferative disorders.

In view of this impressive body of evidence it is not surprising that many attempts have been made to correlate changes in brain polyamine content during development to change in DNA or RNA content. In studies of developing fish [34] or rat [59] brain there was a good correlation between spermine and DNA content and between spermidine and RNA content. It has also been suggested that in rat cerebellum there is a similar developmental pattern of change in polyamines and nucleic acids, especially DNA, whereas in cerebral cortex spermidine correlates with DNA and spermine with RNA [30] though this latter correlation was rather poor. A more detailed study of the regional distribution of polyamines in relation to that of nucleic acids again suggested a correlation of spermidine with RNA except in the medulla, spinal cord and peripheral nerve and a correlation of spermine with DNA, though the spermine: DNA ratio in the brain stem, cerebellum and cord was only half that in diencephalic and telencephalic structures [35]. It must be said that these latter 'correlations' are far from convincing and a study of several bird and mammalian species subsequently failed to reveal any simple relationship between polyamines and nucleic acids [40]. To expect to find a simple numerical relationship between polyamines and nucleic acids in a heterogeneous organ like the brain is, on reflection, perhaps fatuous.

Attempts to relate polyamine distribution to that of protein have been largely unsuccessful. The somewhat tenuous relationship claimed in fish brain [34] was not substantiated by studies on rat brain [30, 35].

Polyamines, being cationic, will bind to any acidic macromolecules including phospholipids. Arguably the most striking relationship seen in studies of the distribution of polyamines is that between spermidine and myelin. Other evidence supports this relationship. Deficiencies of spermidine are seen in the myelin-deficient quaking or jimpy mutant mice. In quaking mice the most marked deficiencies of spermidine are in the hind brain and spinal cord. Deficiencies in the jimpy mutant are less dramatic and are confined to the hind brain [60]. In an autoradiographic study of the distribution of putrescine and its metabolites in rat spinal nerves and ganglia it was found that the labelling was largely confined to the myelin sheath though this was interpreted, almost certainly erroneously, as evidence for binding to a nucleic acid component [61]. Some binding to nuclei was also detected. In this study which investigated the three day period following putrescine administration, the label was present as spermidine and putrescine not as spermine. In brain a second [62] autoradiographic investigation indicated that myelin rich structures were labelled to a smaller extent than grey matter but since this study occupied a 28 day period following the administration of putrescine the label was probably present mainly in spermidine and spermine.

Sucrose density gradient centrifugation reveals binding of added spermidine or spermine to several subfractions of brain tissue but in particular to the nuclear fraction and also to myelin and synaptosomal components of the crude mitochondrial fraction. In fractionated rat hypothalamus [63] binding of spermidine to the nuclear fraction was substantial, accounting for some 60 per cent of the added spermidine. However, in guinea pig cortex only some 20 per cent of added spermidine or spermine was present in the nuclear fraction, the majority being present in the crude mitochondrial fraction. Subfractionation of the endogenous amine content of the crude mitochondrial fraction revealed substantial binding to both myelin and synaptosomes [64]. Experiments using mouse cerebral hemispheres carried out in our laboratories are to a large measure in agreement with this finding. An interesting observation made in guinea pig cortex [64] was that, in a sodium diatrizoate gradient with a balanced ionic composition, binding to myelin was reduced in comparison with that to synaptosomes. After hypo-osmotic shock the polyamines were largely bound to membrane fragments of the synaptosomes. Whereas polyamines bound to myelin could be displaced by the addition of excess polyamine, binding to synaptosome membrane fragments was resistant to displacement. There was some evidence for binding of spermine to synaptic vesicles and spermine or spermidine have been shown to inhibit the binding of acetylcholine, but not of gamma-aminobutyric acid, to synaptic vesicles [65].

NEUROPHARMACOLOGICAL PROPERTIES AND POSSIBLE ROLE IN CENTRAL SYNAPTIC TRANSMISSION

It has generally been assumed that the polyamines have little pharmacological activity and indeed, if their effects on peripheral tissues are considered, this is so. High doses are required before responses in isolated tissues are observed and effects on the central nervous system are only produced by massive doses [66]. However, entry of spermidine and spermine into the central nervous system from the periphery is restricted by a barrier phenomenon [67] and if this is circumvented by the use of intraventricular injection much more dramatic responses can be produced. In mice putrescine had little effect unless it was given in very high doses, but a few microgrammes of spermine or spermidine produced sedation and hypothermia lasting a few hours. Spermine,

which was more potent than spermidine, also produced convulsions. These developed over a period of several hours and the animals became extremely hyperexcitable so that convulsions were precipitated by the slightest sound or touch [68]. Similar effects were produced in rabbits by somewhat larger doses and in addition a marked tachypnoea was noticed. In rabbits, spermine or spermidine given by intraventricular injection produce hyperglycaemia probably by acting on the ventral brain stem [69]. Spermidine is very much less active than spermine in producing convulsions, but high doses produce a pathological syndrome consisting initially of ataxia which appears after 24 hr, but eventually developing over several days into quadriplegia. The animals show anorexia and adipsia and become moribund after about five days. This syndrome is produced by a focal encephalomalacic lesion located in the ventral medulla and involving pyramidal tract destruction. Lesions are also scattered superficially down the spinal cord [68]. A subsequent investigation established that, following intraventricular injection, spermidine is distributed by cerebrospinal fluid and accumulates in the lower brain stem [70], a finding consistent with the locus of the hyperglycaemic effect and of the lesions. A plausible, though unsubstantiated, explanation of the lesioning, is that metabolism to acrolein might be responsible. Whether such lesioning bears any relationship to human disease remains to be established.

The neuropharmacological actions of spermidine and especially of spermine raise the possibility that these substances may modulate or even mediate central synaptic transmission. Possible evidence of a modulator role is provided by the observation that polyamines may activate or inhibit cholinesterase [71] but doses of spermine or spermidine sufficient to produce central effects were without effect on brain acetylcholine content [72] or on that of noradrenaline, dopamine, 5-hydroxytryptamine or gamma-aminobutyric acid.

Putrescine, spermidine and spermine perfused in concentrations up to 10^{-4} M had no effect on the spontaneous activity of the isolated nerve ring of the snail but gamma-aminobutyric acid was also inactive [26]. More recent experiments carried out on rat and cat brain stem neurones have shown that polyamines can influence neuronal firing. In these experiments [73] depression was the commonest response, occurring in some 35 per cent of neurones tested, but this was usually rapid in onset and was always terminated rapidly. Excitation which occurred half as often, developed more slowly but outlasted the period of application and in some neurones firing reached three to five times control levels. Responses to spermine and spermidine were always the same on any one neurone and did not correlate with responses to noradrenaline or acetylcholine. This suggestes that responses to polyamines might be unique to these substances and not mediated through the agency of other neurohumors. Clearly, further studies are appropriate.

For many putative transmitters, density gradient centrifugation has provided useful information in support of a neurotransmitter role, but the presence of multiple binding sites for polyamines in subfractions of brain tissue makes it certain that secondary redistribution will occur during processing and therefore accurate interpretation of the results is impossible. The observation that binding is influenced by the ionic composition of the medium also complicates the issue. Unfortunately the subcellular localisation of the synthetic enzymes is also of little help because these are all soluble. Though most of the ornithine decarboxylase activity and all of the *S*-adenosyl methionine decarboxylase activity present in the crude mitochondrial fraction is present in the synaptosomal subfraction [27] this represents only a part of the total activity.

There is strong evidence to suggest that polyamines are associated with glia in that large increases in brain polyamine content occur in astrocytoma tissue [67] and the increases in scrapie [74] have been attributed to astrocyte hypertrophy. In chick brain spermidine concentration peaked both during gliogenesis and during neuroblast formation [56]. Autoradiographic studies have associated polyamines with both glial [61] and neuronal [62] elements. Some further studies involving electron microscope autoradiography would help to clarify the position.

TRANSPORT

Some years ago it was suggested that polyamines could be transferred from one brain region to another [43] and this was substantiated by our more recent study [70] which concluded that polyamines injected into the cerebral ventricles are taken up by many brain regions and subsequently released and redistributed to other regions by transport in cerebrospinal fluid. Although cerebrospinal fluid polyamine content is increased in acute lymphoblastic leukemia [75] normal levels are very low in comparison with tissue concentrations. Recent work using ventriculo-cisternal perfusion indicates that spermidine and spermine can be cleared from cerebrospinal fluid by an active transport system located in the choroid plexus [76]. This system had a high affinity for both spermidine (K_m 21 μM) and spermine (K_m 24 μM) and spermidine clearance was reduced by spermine and vice versa suggesting that a common carrier is operating.

In mouse cerebral hemispheres a low affinity uptake system for spermidine which was sensitive to temperature and metabolic inhibitors and inhibited by spermine has been described [77]. A similar uptake system for spermine was also present, though at low medium concentrations this was insensitive to temperature or metabolic inhibitors and uptake at these concentrations could well be explained by binding to cellular components. At low concentrations it was also impossible to detect a temperature sensitive component of uptake into synaptosomes [64]. In recent, as yet unpublished, experiments using rat cortex slices, we have characterised a temperature sensitive uptake system with a similar affinity for spermidine to that found in mouse hemisphere slices. Extremely high affinity temperature insensitive component, probably related to binding, were also present. If polyamines were involved in central synaptic transmission an uptake system would probably be necessary for removal of released polyamines since enzymic destruction, being so slow, could not possibly be involved.

Axonal transport may also play a part in the distribution of polyamines. In goldfish optic nerve spermine is extensively transported in the intact nerve but transport decreases in early nerve regeneration. Conversley, spermidine is transported more slowly than spermine in intact nerve but transport increases during regeneration and putrescine is transported only in regenerating nerve [78]. This evidence suggests that spermine is the most likely candidate for a role in neurotransmission whereas putrescine and possibly spermidine might be more important in growth. Pharmacological data supports this in that spermine is the most, and putrescine the least, active of the three substances.

There is some indirect evidence which suggests that polyamines may be released from cerebral cortex tissue in that it has been shown that the spermidine and spermine content of rhesus monkey motor cortex can be decreased by electrical stimulation [39]. Putrescine content was unaffected. Whereas a subsequent study using guinea-pigs failed to confirm this observation or to detect a reduction in cortical polyamine content (or for that matter gamma-aminobutyric acid content) following topical application of solutions rich in potassium, experiments recently undertaken in our laboratories using rabbits have detected a 10 per cent reduction in cortical spermidine and spermine content following a five minute period of stimulation. Following a more subtle stimulus, supramaximal stimulation of the median nerve of the fore limb, a small but statistically insignificant reduction in the spermine content of the contralateral cortex was produced, whereas spermidine content was unchanged. These experiments are, to say the least, equivocal, but they do provide encouragement for further study. Clearly the more direct approach of measuring the released amine, rather than the amount remaining in the tissue, is required and such studies are being actively pursued in these laboratories at present with promising results.

BEHAVIOURAL STUDIES

Although polyamines in brain are not labile their concentrations may be changed over a period of several weeks and such changes may parallel behavioural change in the animal. Thus, Japanese investigators have claimed that in mice made aggressive by isolation, there is an increase in brain spermidine content which parallels the development of aggression. This response does not occur in non-aggressive isolated mice [79]. Return of aggressive isolated mice to grouped housing, which renders them non-aggressive, reversed the increase in spermidine content [80]. A small increase in brain spermine content was reported in isolated aggressive mice but much larger increases were produced in animals made aggressive by electro-shock [79]. The same group have also reported regional changes in rat brain polyamine content following bilateral destruction of the olfactory bulbs [81]. Confirmation by other groups of these findings is awaited.

CONCLUSION

Despite their long history, the attention given to polyamines is meagre in comparison with that lavished on comparable substances present in the brain. With the present level of specialisation in neuroscience it is all too easy to disregard developments which are not directly related to one's own restricted sphere of interest. The objective of the present commentary has been to draw attention to these fascinating substances and to point out areas in which the application of freely available techniques should provide answers to important questions.

It is far too early to draw any firm conclusions about the rôle of these substances in central nervous function but a rôle in brain growth during early development, or following injury, seems likely. This rôle might be mediated by interactions with nucleic acids. Such a function does not preclude other rôles for polyamines in the nervous system, and spermine is perhaps the most likely candidate for a modulator or transmitter function. It is the conviction of the author that further study of these amines is well worthwhile and his hope that this article will help to provide the necessary stimulus.

REFERENCES

1. H. Tabor and C. W. Tabor, *Pharmac. Rev.* **16**, 245 (1964).
2. E. J. Herbst and U. Bachrach, *Ann. N.Y. Acad. Sci.* **171**, 691 (1970).
3. S. S. Cohen, *Introduction to the Polyamines*, Prentice-Hall, New Jersey (1971).
4. D. H. Russell (Ed.), *Polyamines in Normal and Neoplastic Growth*, Raven, N.Y. (1973).
5. R. A. Campbell, D. R. Morris, D. Bartos, G. D. Daves and F. Bartos, (Eds), *Advances in Polyamine Research* **1**, Raven, N.Y. (1978).
6. R. A. Campbell, D. R. Morris, D. Bartos, G. D. Daves and F. Bartos, (Eds), *Advances in Polyamine Research* **2**, Raven, N.Y. (1978).
7. A. Raina and J. Janne, *Med. Biol. Helsinki* **53**, 121 (1975).
8. G. Litwack and S. R. Rosenfield, *Biochem. biophys. Res. Commun.* **52**, 181 (1973).
9. S. R. Butler and S. M. Schanberg, *Life Sci.* **18**, 759 (1976).
10. T. R. Anderson and S. M. Schanberg, *J. Neurochem.* **19**, 1471 (1972).
11. Y. Friedman, S. Park, S. Levasseur and G. Burke, *Biochim. biophys. Acta* **500**, 291 (1977).
12. U. Bachrach, *Proc. natn. Acad. Sci. U.S.A.* **72**, 3087 (1975).
13. S. H. Snyder, E. G. Shaskan and S. Harik, in *Polyamines in Normal and Neoplastic Growth* (Ed. D. H. Russell) p. 199. Raven, N.Y. (1973).
14. T. R. Anderson and S. M. Schanberg, *Biochem. Pharmac.* **24**, 495 (1975).
15. L. J. Roger, *Endocrinology* **95**, 904 (1974).
16. M. E. Lewis, J. Lakshmanan, K. Nagaiah, P. C. MacDonnell and G. Guroff, *Proc. natn. Acad. Sci. U.S.A.* **75**, 1021 (1978).
17. L. J. Roger and R. E. Fellows, *Fedn Proc.* **34**, 254 (1975).
18. S. R. Butler and S. M. Schanberg, *Biochem. Pharmac.* **24**, 1915 (1975).
19. T. A. Slotkin, C. Lau and M. Bartolomé, *J. Pharmac. exp. Ther.* **199**, 141 (1976).
20. P. V. Thadani, C. Lau, T. A. Slotkin and S. M. Schanberg, *Biochem. Pharmac.* **26**, 523 (1977).
21. P. V. Thadani, C. Lau, T. A. Slotkin and S. M. Schanberg, *Neuropharmacology* **16**, 289 (1977).
22. S. R. Butler and S. M. Schanberg, *Life Sci.* **21**, 877 (1977).
23. S. R. Butler, M. R. Suskind and S. M. Schanberg, *Science, N.Y.* **199**, 445 (1978).
24. P. Kleihues, K. A. Hossmann, A. Pegg, K. Kobayashi and V. Zimmerman, *Brain Res.* **95**, 61 (1975).

25. A. E. I. Pajunen, O. A. Hietala, E. L. Virransalo and R. S. Piha, *J. Neurochem.* **30**, 281 (1978).
26. R. M. Gould and G. A. Cottrell, *Comp. Biochem. Physiol.* **48B**, 591 (1976).
27. G. L. Schmidt and G. L. Cantoni, *J. Neurochem.* **20**, 1373 (1973).
28. J. A. Sturman and G. E. Gaull, *J. Neurochem.* **25**, 267 (1975).
29. E. G. Shaskan and J. M. Marshall, *Fedn Proc.* **32**, 429 (1973).
30. E. G. Shaskan, J. H. Haraszi and S. H. Snyder, *J. Neurochem.* **20**, 1443 (1973).
31. H. G. Williams-Ashman, A. Corti and B. Tadolini, *Ital. J. Biochem.* **25**, 5 (1976).
32. J. A. Sturman, *Life Sci.* **18**, 879 (1976).
33. A. Raina and P. Hannonen, *FEBS Lett.* **16**, 1 (1971).
34. N. Seiler and U. Lamberty, *J. Neurochem.* **20**, 709 (1973).
35. N. Seiler and T. Schmidt-Glenewinkel, *J. Neurochem.* **24**, 791 (1975).
36. S. Harik and S. H. Snyder, *Brain Res.* **66**, 328 (1974).
37. E. G. Shaskan, *J. Neurochem.* **28**, 509 (1977).
38. G. G. Shaw and A. J. Pateman, *J. Neurochem.* **20**, 1225 (1973).
39. D. H. Russell, E. Gfeller, L. J. Marton and S. Le Gendre, *J. Neurobiol.* **5**, 349 (1974).
40. N. Seiler and U. Lamberty, *Comp. Biochem. Physiol.* **52B**, 419 (1975).
41. H. Shimizu, Y. Kakimoto and I. Sano, *J. Pharmac. exp. Ther.* **143**, 199 (1964).
42. D. H. Russell, V. J. Medina and S. H. Snyder, *J. biol. Chem.* **245**, 6732 (1970).
43. E. G. Shaskan and S. H. Snyder, *J. Neurochem.* **20**, 1453 (1973).
44. Y. Kakimoto and T. Nakajima, *J. biol. Chem.* **244**, 6003 (1969).
45. T. L. Perry, S. Hansen and M. Kloster, *J. Neurochem.* **19**, 1395 (1972).
46. N. Imaoka and Y. Matsuoka, *J. Neurochem.* **22**, 859 (1974).
47. B. W. Lines and D. R. Morris, *Biochim. biophys. Acta* **228**, 223 (1971).
48. C. M. Calderara, M. S. Morruzzi, C. Rossoni and B. Barbiroli, *J. Neurochem.* **16**, 309 (1969).
49. T. Nakajima, *J. Neurochem.* **20**, 735 (1973).
50. L. T. Kremzner, J. M. Hiller and E. J. Simon, *J. Neurochem.* **25**, 889 (1975).
51. C. A. Halliday and G. G. Shaw, *J. Neurochem.* **26**, 1199 (1976).
52. J. A. Sturman, N. A. Ingoglia and T. D. Lindquist, *Life Sci.* **19**, 719 (1976).
53. R. A. Alarcon, *Archs Biochem. Biophys.* **137**, 365 (1970).
54. N. Seiler, M. W. Wiechman, H. A. Fischer and G. Werner, *Brain Res.* **28**, 317 (1971).
55. N. Seiler, M. J. Al-Therib and K. Katuoka, *J. Neurochem.* **20**, 699 (1973).
56. K. Sobue and T. Nakajima, *J. Neurochem.* **30**, 277 (1978).
57. A. F. Reynolds and D. H. Russell, *Fedn Proc.* **32**, 429 (1973).
58. P. P. Giorgi, *Biochem. J.* **120**, 643 (1970).
59. N. Seiler and U. Lamberty, *J. Neurochem.* **24**, 5 (1975).
60. D. H. Russell and H. Meier, *J. Neurobiol.* **6**, 267 (1975).
61. H. A. Fischer, M. Schroder and N. Seiler, *Z. Zellforsch.* **128**, 393 (1972).
62. H. A. Fischer, H. Koor, N. Seiler and G. Werner, *Brain Res.* **39**, 197 (1972).
63. I. A. Michaelson and H. R. Smithson, *Biochem. Pharmac.* **20**, 2091 (1971).
64. N. Seiler and K. Deckardt, *Neurochem. Res.* **1**, 469 (1976).
65. K. Kuriyama, E. Roberts and J. Vos, *Brain Res.* **9**, 231 (1968).
66. G. G. Shaw, *Archs int. Pharmacodyn. Thér.* **198**, 36 (1972).
67. L. T. Kremzner, R. E. Barrett and M. J. Terrano, *Ann. N.Y. Acad. Sci.* **171**, 735 (1970).
68. D. J. Anderson, J. Crossland and G. G. Shaw, *Neuropharmacology* **14**, 571 (1975).
69. D. J. Anderson and G. G. Shaw, *Br. J. Pharmac.* **52**, 205 (1974).
70. C. A. Halliday and G. G. Shaw, *J. Neurochem.* **26**, 1199 (1976).
71. R. Anand, M. G. Gore and G. A. Kerkut, *J. Neurochem.* **27**, 381 (1976).
72. G. G. Shaw, *Biochem. Pharmac.* **26**, 1450 (1977).
73. M. A. Wedgwood and J. H. Wolstencroft, *Neuropharmacology* **16**, 445 (1977).
74. P. P. Giorgi, E. J. Field and G. Joyce, *J. Neurochem.* **19**, 255 (1972).
75. O. M. Rennert, D. L. Lawson, J. B. Shukla and T. D. Miale, *Clinica chim. Acta* **75**, 365 (1977).
76. C. A. Halliday and G. G. Shaw, *J. Neurochem.* **30**, 807 (1978).
77. A. J. Pateman and G. G. Shaw, *J. Neurochem.* **25**, 341 (1975).
78. N. A. Ingoglia, J. A. Sturman and R. A. Fisner, *Brain Res.* **130**, 433 (1977).
79. T. Tadano, M. Onoki and K. Kisara, *Folia pharmac. jap.* **70**, 9 (1974).
80. T. Tadano, *Folia pharmac. jap.* **70**, 457 (1974).
81. T. Sakurada, M. Imai, T. Tadano and K. Kisara, *Jap. J. Pharmac.* **26**, 509 (1976).