NOREPINEPHRINE AS A CENTRAL SYNAPTIC TRANSMITTER

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THE ULTIMATE test of the function of central noradrenergic synapses must result from the selective activation of these terminals and from the detection of their activity patterns in the unrestrained experimental animal. That such higher order testing can even be conceived indicates the conceptual and experimental advances which have occurred since the last major catecholamine review (SALMOIRAGHI, 1966; BLOOM, 1968; BLOOM *et al.*, in press; HOFFER and BLOOM, 1972). At that time emphasis was rightly placed upon data which demonstrated that iontophoretically administered norepinephrine (NE) could produce actions with sufficient potency and regularity to merit consideration as a synaptic transmitter. At present, many differences in interpretation are still associated with data obtained by applying the iontophoretic method to CNS areas in which the precise anatomy of the NE-containing terminals has yet to be related to the cells undergoing testing. Beyond these interpretive problems however, it has been possible to determine the actions of NE, fibre systems arising from the pontine nucleus, locus coeruleus (LC), and projecting to the cerebellar Purkinje cells (HOFFER *et al.*, 1971a) and to the hippocampal pyramidal cells (SEGAL and BLOOM, in press) and to record from the LC neurons themselves during unrestrained observations of sleeping and waking behaviour (CHU and BLOOM, 1973). All of these actions which reflect upon the function of central noradrenergic synapses have been recently reviewed, (BLOOM *et al.*, in press; HOFFER and BLOOM, 1973; HOFFER *et al.*,1971a) and in the space allotted for the present survey we will concentrate upon two questions: what are the principal effects of iontophoretically applied NE; what is the nature of the physiological central NE receptor and the molecular mechanisms by which NE synapses can exert these effects.

Principle actions of iontophoretically applied NE. The extensive work of the past 5 years has amply supported the view that NE is able to effect the discharge of neurons in all portions of the neuraxis (see Table 1 of HOFFER and BLOOM, 1973). Present work is more realistically directed at determining the functional significance of the presence or absence of responses to NE and the qualitative nature of the response, i.e., excitatory or inhibitory. Retrospective analysis of experimental inconsistencies suggests that major variables were uncontrolled. Thus, excitatory responses to NE in the cerebral cortex were found to be more frequent when the animal was unanesthetised or under the influence of halothane anesthesia while inhibitory cortical responses were more frequent with barbiturate anesthesia (JOHNSON *et al.*, 1969). Similarly, the pH of the NE solution to be iontophoretically applied also takes on critical importance, as pH values less than 4 are associated with major increases in the frequency of excitatory NE responses (FREDERICKSON *et al.*, 1972). An extreme view of the anesthesia-pH controversy is that all excitatory responses to NE are the result

of vasoconstriction artefacts (STONE, 1971), a suggestion which was quickly rejected (BOAKES *et al.*, 1972).

The eclectic view of these controversies must await additional observations on several other factors such as the cytological or functional heterogeneity of the cell population being tested: it is known that when defined poulations of neurons are tested, the responses are far more reproducible than when all randomly encountered neurons in a given CNS region are lumped together (SALMOIRAGHI and STEFANIS, 1967). An additional cytological index which must be considered is whether or not the population of cells to be tested receives a demonstrable synaptic input of NE-containing terminals. Thus, the highly variable responsiveness of hypothalamic neurons to NE (BLOOM *et al.*, 1963) reduces to clear cut patterns of primarily excitatory responses when the data are restricted to those border cells of the ventro-medial nucleus which receives NE-containing synapses (KREBS and BENDRA, 1971). The same degree of variable results can also be obtained on random populations of medullary neurons, and the data here reduce to primarily inhibitory actions when analysis is restricted to cells identified as cranial motoneurons (OLIVER *et al.*, 1972) all of which are the recipient of relatively dense NE-containing terminals. The latter two observations indicate that even when anesthesia and solution pH are held constant, variable results can still be obtained when no significance is attached to the neuron to be tested or the input it receives.

When we restrict our condensation of reported results to those populations of defined post-synaptic cells for which NE-containing nerve terminals have been demonstrated by light or electron microscopic techniques, the somewhat shorter list obtained shows almost universally inhibitory results (BLOOM, 1968; BLOOM *et al.*, in press; HOFFER and BLOOM, 1972; SALMOIRAGNI and STEFANIS, 1967). Thus, cerebellar Purkinje cells, (BLOOM *et al.*, in press; HOFFER and BLOOM, 1972; HOFFER *et al.*, 1971a) olfactory bulb mitral cells, (BLOOM *et al.*, 1964) supraoptic and paraventricular hypothalamic neurosecretory neurons (BARKER *et al.*, 1971), spinal motoneurons (WRIGHT and SALMOIRAGNI, 1967), primary neurons of the medial geniculate (TEBECIS, 1970) and polysensory neurons of the primate frontal cortex (NELSON *et al.*, in press) show reproducible inhibitory effects, in addition to the populations of defined neurons mentioned above in the hypothalamus (BLOOM *et al.*, 1963) and medulla (KREBS and BENDRA, 1971). The most serious obstacle to interpretation of even these results has been that the NE-containing pathway has not been amenable to selective electrical activation for comparisons of qualitative and pharmacological results on the specified post-synaptic population of test neurons. Conversely, synaptic effects which were subjectible to selective activation were neither exclusively NE-containing nor sensitive to NE antagonists. Thus, in neither the olfactory mitral cells (SALMOIRAGNI *et al.*,1964) nor the hypothalamic neurosecretory cells (NICOLL and BARKER, 1971) could the recurrent antidromic synaptic inhibition be removed by the results of acute or chronic NE depletion, or NE receptor blockade.

Over the past several years we have pursued the mechanism by which NE slows the discharge of cerebellar Purkinje cells (HOFFER *et al.*, 1971b). We have used light and electron microscopy to establish that these cells receive NE-containing synapses onto their dendrites (BLOOM *et al.*, 1971), and that these NE fibres arise from the LC (OLSON and FUXE, 1971; BLOOM *et al.*, 1972a). By electrophysiological methods, we have analysed the pharmacological receptors of the Purkinje cells (SIGGINS *et al.*,

1971a) and the effects of electrical activation of the pathway (HOFFER *et al.*, 1971a; SIGGINS *et al.*, 1971b). Briefly, these experiments indicate that NE slows Purkinje cells by interaction with a beta receptor, by prolonging the pauses between bursts of single spikes without effect on climbing fibre responses. By intracellular recordings, NE hyperpolarises the membrane of Purkinje cells and this hyperpolarisation is generally accompanied by increased membrane resistance (but never by increased membrane conductance). The actions of norepinephrine on the Purkinje cell are blocked by iontophoretic application of a beta antagonist (MJ 1999), of prostaglandins of the E series and by nicotinate; the latter both inhibit adenylate cyclase in some autonomic tissues. The effects of norepinephrine on discharge rate and membrane parameters are precisely emulated by iontophoretic application of cyclic AMP, and the effects of both the applied cyclic AMP and of NE are potentiated by any of several phosphodiesterase inhibitors. On the basis of these data, we proposed (HOFFER *et al.*,1971b; SIGGINS *et al.*, 1971a) that the synaptic action of NE was mediated by an interaction via the adenyl cyclase of the cerebellar cortex, known to be highly responsive to NE (see BLOOM *et al.*, in press).

With the anatomical information that the cerebellar NE fibres arose from the LC (OLSON and FUXE, 1971; BLOOM *et al.*, 1972a), it was possible to test this proposal by activating and analysing the effects of the pathway on Purkinje cell properties. These experiments disclosed that stimulation of the pathway inhibited Purkinje cell discharge, especially single spike bursts, that the inhibitory effects of stimulating LC required active synthesis of NE, and that no effects on cerebellar neuronal discharge were observed when the area of the locus was stimulated in animals pretreated with 6-hydroxydopamine to eradicate the adrenergic projection to the cerebellum (HOFFER *et al.*, 1971a). By intracellular recording during the activation of the LC, Purkinje cells were found to be hyperpolarised and this hyperpolarisation was usually accompanied by a definitive increase in the resistance of the membrane (SIGGINS *et al.*, 1971b). Similar effects of NE have been observed on motoneurons (ENGBORG and MARSHALL, 1971). Pharmacologically, activation of the LC led to an inhibition of spontaneous discharge which could be potentiated by local iontophoresis of phosphodiesterase inhibitors onto the Purkinje cell and could be blocked by local iontophoretic administration of prostaglandins of the E series. All these results supported the concept that this adrenergic projection could be operating by the trans-synaptic elevation of cyclic AMP in Purkinje cells. The latter observation has now been documented by application to tissue sections of an immunocytochemical method for cyclic AMP (BLOOM *et al.*, 1972b). Using this method we have observed that topical application of NE or electrical activation of the LC will elevate the number of Purkinje cells showing positive immunocytological staining for cyclic AMP from resting frequencies of 5–15 per cent to levels greater than 75 per cent (SIGGINS *et al.*, 1973). Neither topical application of GABA, glycine, histamine, or acetylcholine, or electrical activation of other cerebellar pathways has this effect on Purkinje cell cyclic AMP (SIGGINS *et al.*, 1973). A generally similar set of observations, although less extensively analysed have been obtained by Greengard and his co-workers for, the dopaminergic intraganglionic synapses in rabbit sympathetic ganglia (GREENGARD, *et al.*, 1973).

Finally, it is worthwhile considering whether the paradigm of NE effects at the synaptic receptor examined on cerebellar Purkinje cells is to be considered as a

general example of the central synaptic actions of NE or rather as a specific and somewhat unique type of receptor. Based on biochemical experiments, it is known that the actions of transmitters on adenylate cyclase activation are specific, within a species, for certain regions of the brain, and within regions, for certain transmitters (RALL, 1971). In the cerebellum of the rat, NE is the most active transmitter substance in activating cyclase activity (RALL, 1971) but in the cerebral cortex, NE-induced adenylate cyclase activation is highly species dependent, being relatively poor in rat and quite potent in primate (SKOLNICK *et al.*, in press) and human cortex (FUMAGILLI *et al.*, 1971; SHIMAZU *et al.*, 1971).

Viewed from a different perspective, we may ask two testable questions: (1) need all actions of NE be explicable in terms of activation of cyclic AMP synthesis and the subsequent actions of the cyclic nucleotide; (2) are all actions of cyclic AMP at central synapses due to activation of the adenylate cyclase by catecholamines. The first action might be tested by analysing whether the ability of NE to affect neuronal discharge correlates with the ability of NE to activate adenylate cyclase of neurons in a given region of a given species. Such a test has been made by Phillis and collaborators (JORDAN *et al.*, 1972) who found that the actions of NE were uniformly inhibitory across species lines regardless of the neurochemically determinable stimulatory potency of NE on the adenylate cyclase. On the other hand, the squirrel monkey shows marked receptivity to NE (NELSON *et al.*, in press) and a very striking elevation of cyclic AMP synthesis in vitro to NE (SKOLWICK *et al.*, in press). Part of the solution to these apparently paradoxical data may depend upon the same considerations with which we began this survey, namely that receptivity to NE may not be assumed to infer the presence of NE-mediated synapses. In fact, a large number of NE terminals in an area might even result in apparent poor receptivity due to rapid accumulation of iontophoretically released NE enroute to post-synaptic receptors. If that assumption be granted, then clearly receptivity to NE also need not imply an NE-sensitive adenylate cyclase underlying the action. Furthermore, the ability to demonstrate the capacity of a putative transmitter to activate adenylate cyclase activity is also in a stage of continual technical development, in which co-factors and ionic conditions are extremely important for the result observed. Therefore, when faced with a negligible apparent rise in the cyclic AMP synthesis rate *in vitro* for a brain slice, one must consider that the system being used to test for the rise in cyclase activity may have been inadequate or that the cells within the slice which exhibited a stimulatory effect by the exogenous transmitter represented such a small proportion of the total tissue mass of the slice that the rise went undetected by the assay. In the case of the cerebellar cortex, the use of the immunocytochemical staining method offers a direct demonstration of increased cyclic AMP content of Purkinje cells, a system where the synapses and the effects of the synaptic pathway can be equated with the action of applied NE. For other regions of the brain, particularly where the cytology is less amenable to resolution of precise synaptology to cells identifiable during extracellular recording, these same tests may be difficult to complete.

It has also been observed that the actions of NE in the cerebral cortex (JORDAN *et al.*, 1972) and in the brainstem (ANDERSON *et al.*, 1973) does not show clearcut potentiation when phosphodiesterase inhibitors are administered either iontophoretically or parenterally. LAKE *et al.* (1972) did in fact find that aminophylline and

papaverine could potentiate the inhibitory actions of NE in approximately 70% of the unidentified neurons they tested in the feline cerebral cortex. However, these investigators interpreted this response to be a non-specific summation of inhibitory actions since the phosphodiesterase inhibitors were directly inhibitory on the same cells. While these results may be interpreted as non-specific, it perhaps should be expected that phosphodiesterase inhibition—which would be expected to elevate cyclic AMP levels generally—might produce more widespread actions than NE, particularly since the action of phosphodiesterase is independent of the hormone which activated cyclic AMP synthesis. Both of these considerations should also be weighed in evaluation of the observations on prostaglandin antagonisms of NE responsiveness in the cat cortex (JORDAN *et al.*, 1972) and the cat brainstem (ANDERSON *et al.*, 1973).

Perhaps it would be most fair to ask just what evidence would be needed to determine that the actions of NE in a given region were unrelated to an effect on adenylate cyclase. Given the limitations imposed by present techniques, one would have to demonstrate the following: (1) NE synapses, arising from a defined NE cell group, are present on cells electrophysiologically identifiable during electrical recording; (2) the action of NE and of the NE-pathway is not emulated by cyclic AMP, either on the rate or pattern of discharge or on membrane properties; (3) the actions of phosphodiesterase inhibitors do not potentiate the NE-related actions, but do selectively potentiate the actions of exogenous cyclic AMP; (4) the biochemical or immunocytochemical estimation of cyclase activity is independent of the actions of applied NE or activation of the NE synapses.

In conclusion, despite the great progress which has been made in the electrophysiological documentation of NE as a central synaptic transmitter in selected regions of the CNS, many technical and interpretive pitfalls lie in the path of experiments designed to extend these observations and approaches to other CNS regions. A fruitful approach may be difficult to obtain, but still it must be sought.

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REFERENCES

ANDERSON E: G., HAAS H. L. and HOSLI L. (1973) *Brain Res.* **49,** 467.
BARKER J. L., CRAYTON J. C. and NICOLL R. A. (1971) *J. Physiol.* **218,** 19.
BLOOM F. E. (1968) In: *Psychopharmacology* (EFFRON D. Ed.) p. 355. Government Printing Office, Washington.
BLOOM F. E., OLIVER A. P. and SALMOIRAGHI G. C. (1963) *Int. J. Neuropharmac.* **2,** 181.
BLOOM F. E., COSTA E. and SALMOIRAGHI G. C. (1964) *J. Pharmac. exp. Ther.* **146,** 16.
BLOOM F. E., HOFFER B. J. and SIGGINS G. R. (1971) *Brain Res.* **25,** 501.
BLOOM F. E., HOFFER B. J. and SIGGINS G. R. (1972a) *Biol. Psychiat.* **4,** 157.
BLOOM F. E., HOFFER B. J., BATTENBERG E. F., SIGGINS G. R., STEINER A. L., PARKER C. W. and WEDNER H. J. (1972b) *Science* **177,** 436.
BLOOM F. E., CHU N-S., HOFFER B. J., NELSON C. N. and SIGGINS G. R. (1973) *Neurosci. Res.* **5,** 53.
BOAKES R., BRADLEY P., CANDY J. and DRAY A. (1972) *Nature, New Biol.* **239,** 151.
CHU N-s. and BLOOM F. E. (1973) *Science* **179,** 908.
ENGBORG I. and MARSHALL K. C. (1971) *Acta. physiol. scand.* **83,** 142.
FREDERICKSON R., JORDAN L. and PHILLIS J. (1972) *Brain Res.* **35,** 556.
FUMAGILLI R., BERNAREGGI V., BERTI F. and TRABUCCHI M. (1971) *Life Sci.* **10,** 1111.
GREENGARD P., KEBABIAN J. W. and MCAFEE D. A. (1973) In: *Proc. Vth Int. Congr. Pharmac.* (ACHESON G Ed.), Karger, Basel.

HOFFER B. J. and BLOOM F. E. (1973) In: *The Influence of the Limbic System on Autonomic Function* (HOCHMAN C. Ed.) p. 91. Charles Thomas, Springfield. (in press).
HOFFER B. J., SIGGINS G. R., OLIVER A. P. and BLOOM F. E. (1971a) *J. Pharmac. exp. Ther.* **184,** 553.
HOFFER B. J., SIGGINS G. R., OLIVER A. P. and BLOOM F. E. (1971b) *Ann. N.Y. Acad. Sci.* **185,** 531.
JOHNSON E., ROBERTS M. and STRAUGHAN D. (1969) *J. Physiol.* **203,** 261.
JORDAN L. M., LAKE N. and PHILLIS J. (1972) *Europ. J. Pharmac.* **20,** 381.
KREBS H. and BENDRA B. D. (1971) *Nature, Lond.* **229,** 178.
LAKE N., JORDAN L. M. and PHILLIS J. (1972) *Nature, New Biol.* **240,** 249.
NELSON C., HOFFER B. J., CHU N-s. and BLOOM F. E. *Brain Res.* (in press).
NICOLL R. A. and BARKER J. L. (1971) *Brain Res.* **35,** 501.
OLIVER A. P., SIMS K. L. and BLOOM F. E. (1972) *Abst. Vth Int. Congr. Pharmac.* 171.
OLSON L. and FUXE K. (1971) *Brain Res.* **28,** 165.
RALL T. W. (1971) *Ann. N.Y. Acad. Sci.* **185,** 520.
SALMOIRAGHI G. C. (1966) *Pharmac. Rev.* **18,** 717.
SALMOIRAGHI G. C. and STEFANIS C. (1967) *Int. Rev. Neurobiol.* **10,** 1.
SALMOIRAGHI G. C., COSTA E. and BLOOM F. E. (1964) *Am. J. Physiol.* **207,** 1417.
SEGAL M. and BLOOM F. E. *Abst. 3rd Ann. Meet. Soc. Neurosci.* (in press).
SHIMIZU H., TANAKA S., SUZUKI T. and MATSUKADO Y. (1971) *J. Neurochem.* **18,** 1157.
SKOLNICK P., HUANG M., DALY J. and HOFFER B. J. (1973) *J. Neurochem.* (in press).
SIGGINS G. R., HOFFER B. J. and BLOOM F. E. (1971a) *Brain Res.* **25,** 535.
SIGGINS G. R., HOFFER B. J., OLIVER A. P. and BLOOM F. E. (1971b) *Nature, Lond.* **233,** 481.
SIGGINS G. R., BATTENBERG E. F., HOFFER B. J., BLOOM F. E. and STEINER A. L. (1973) *Science* **179,** 585.
STONE T. (1971) *Nature, Lond.* **234,** 145.
TEBECIS A. (1970) *Neuropharmac.* **9,** 381.
WEIGHT F. F and SALMOIRAGHI G. C. (1967) *Nature, Lond.* **213,** 1229.