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B. CENTRAL ADRENERGIC SYNAPSES

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There is now much indirect evidence suggesting a synaptic transmitter function of norepinephrine (NE) and, perhaps, other catecholamines, in the mammalian central nervous system. The evidence consists of 1) the existence of regional differences in the central nervous system distribution of catecholamines and their metabolizing enzymes (11, 39, 58), 2) the presence of catecholamines in higher concentration in fractions of brain homogenates that are rich in synaptic terminals (28, 38, 40), 3) the occurrence of dark-core vesicles (7, 28, 42) within some central axon-terminals, similar to those found in peripheral adrenergic nerve endings (29, 45, 46), 4) the histochemical localization of induced fluorescence attributable to catecholamines in perikaria and "terminals" of central neurons (27, 31), and 5) certain pharmacological studies, reviewed here, which have made use of the technique of electrophoretic administration of chemical substances in the immediate proximity of individually recorded central neurons ("microelectrophoresis," (17); "micro-iontophoresis," (35)).

The traditional pharmacological approach has been to study changes in the EEG, unit activity, reflexes, and behavior resulting from the intravenous, intra-arterial or intraventricular injection of catecholamines and related drugs (*cf.* 14, 16, 33, 39, 47, 51). These types of observation are subject to interpretative difficulties because of the very large number of potential sites of drug action. When a dependence of the observed change in neural activity from concomitant circulatory, metabolic, and peripheral factors can be excluded, the occurrence of such a change is suggestive of a central site of drug action. However, given the multiplicity and complexity of synaptic inputs on any given central neuron and the speed at which transmission occurs, it is clear that this methodological approach usually cannot provide evidence directly relatable to synaptic activity of any particular neuron. For this reason, this type of data will not be reviewed here.

To meet the electrophysiological and pharmacological criteria (*cf.* 30, 41) suggested for transmitter identification, it would be necessary to record intracellularly from a given central neuron while administering the suspected transmitter and related drugs directly outside its membrane. With this approach, a comparison could be made of the membrane conductance changes resulting from the administration of the suspected transmitter with those induced by stimulation of the relevant synaptic input and of the effects on both of locally administered potentiating and blocking agents. This kind of evidence is as yet unavailable for any of the substances currently suspected as central synaptic transmitters but its realization is now technically feasible.

THE TECHNIQUE OF MICROELECTROPHORESIS

This technique utilizes the property of ions to move in an electrical field and relies upon the use of multibarreled glass micropipette electrodes to administer

drugs directly at the site of unit recording. It has the merit of by-passing the major diffusional and enzymatic barriers and of greatly limiting the number of potential sites of drug action.

In the configuration most often used, 5 glass tubings are fused together and drawn to a common tip of 3 to 10 μ overall diameter, with separate orifices for each of the 5 channels, permitting independent administration by electrophoresis of up to 4 drugs directly at the site of recording. With this instrument, only extracellular recording from the tested unit is possible, since all orifices at the tip are in the same plane.

Intracellular recording and extracellular drug administration are possible with co-axial microelectrodes (22), consisting of two micropipettes concentrically arranged so that the tip of the inner micropipette protrudes 40 to 60 μ from the tip orifice of the outer micropipette. Because of the difficulties inherent in intracellular recordings in the mammalian central nervous system and because only one drug at the time can be tested with this instrument, its full utilization is limited to those relatively rare instances in which there is a fair chance of sufficiently prolonged intracellular recordings and in which the kind of drug that would be critical for the testing of a hypothesis is already known, as was the case in studies on the effects of certain amino acids on motoneurons (22, 23, 25, 26). Nevertheless, routine use of concentric electrodes may prove desirable when the small size of the neuron under investigation requires the use of an ultrafine microelectrode for its detection and for the extracellular recording of its activity or when it may be informative to apply a drug at some distance from the recording site, as would be the case with neurons possessing an extensive dendritic tree of known spatial configuration.

Lastly, it will be noted that there are no technical barriers to the development of an instrument that would combine the essential features of 5-barreled and concentric microelectrodes (*i.e.*, an instrument in which the ultrafine tip of a recording micropipette protrudes from the central orifice of a multiple-barreled glass microelectrode) if the opportunity for its use were to arise.

REGIONAL DISTRIBUTION OF CATECHOLAMINE-SENSITIVE NEURONS

Most catecholamine studies by microelectrophoresis have dealt with neuron responses to norepinephrine (NE). These have been investigated in the cat's cortex (36, 37), lateral geniculate (18, 19), hippocampus (3, 56), red nucleus, septum and dentate gyrus (56), caudate nucleus (5), hypothalamus (6), medulla (9, 10, 21), Deiters nucleus (63), cerebellar flocculus (63), and lumbar segments of the spinal cord (18, 24, 53, 61), as well as in the rabbit olfactory bulb (4, 50, 60). Sensitivity of individual neurons to dopamine has been tested in the cat's cortex (37), caudate nucleus (5), lateral geniculate (19), ventrobasal thalamic complex (2) and lumbar segments of the spinal cord (15); to E only in the cat's cortex (37). Several chemical analogues of these drugs have been compared on neurons in the lateral geniculate (19) and the cortex (37).

The general picture that emerges from these studies can be summarized as follows. 1) In all regions thus far explored there are some neurons that are

sensitive to the administration of NE. Earlier reports on the absence of NE-sensitive neurons in the lumbar segments of the spinal cord (24) and the medulla (21) are discounted by recent studies made in this (53, 61) and other (9, 10) laboratories. Dopamine-sensitive neurons also are found. In the caudate, when the neuron is sensitive to both NE and dopamine and the response is in the same direction, the response to NE is usually greater for equal values of electrophoretic current used to eject NE and dopamine from the microelectrode (5). 2) In any one region only some neurons are sensitive to the test substance, but the proportion of sensitive to insensitive units varies in different regions. Similarly, sensitivity to the test substance of individual neurons within the same region (as judged from the strength and the duration of the ejecting current required to induce a clear response) varies over a wide range but responsive units in certain regions (*e.g.*, the hippocampus) as a group are more sensitive to NE than units in other regions (*e.g.*, the cortex). 3) The same catecholamine can cause either facilitation or depression of unit activity depending upon the type of neuron being tested.

Coupled with the evidence provided by biochemical studies (11, 39, 59) and by histochemical findings with fluorescence microscopy (31) showing inter- and intraregional differences in the distribution of catecholamine-containing nerve fibers and perikaria, the observations summarized above could be construed as supportive evidence for the presence of central adrenergic synapses in the mammalian central nervous system. In fact, neurons receiving this type of synapse would be expected to show responsiveness to the locally administered catecholamine, while those devoid of these synapses would be expected to be insensitive. Additional supportive evidence is provided by the observation (53) that the proportion of NE-sensitive units among a population of nerve cells of the same functional type (*e.g.*, pyramidal tract neurons, hippocampal pyramidal layer neurons, Deiters nucleus neurons, Renshaw cells, and mitral cells) is much greater than that of randomly tested neurons in the same region. This finding would be expected if it were assumed as likely that neurons of a given functional type in the same region share in large measure the same type of synaptic input, thus possessing essentially the same types of synaptic receptor.

It should be borne in mind, however, that the pharmacological observations supportive of this line of reasoning must be interpreted with caution. Quantification of inter- and intraregional differences in unit sensitivity to a test substance is made difficult by the many technical and biological factors that can affect the results, such as the size, recording and current-carrying characteristics of the microelectrode used, the care with which all recordable units are tested, the presence or absence of anesthesia, the type of surgery used, the degree of facilitation or depression of unit activity prevailing at the time of testing, *etc.* These variables tend in the main to cause falsely "negative" results, *i.e.*, evidence for unresponsiveness. It is imperative in this type of study to offset the influence of the many variables that can affect unit responsiveness through repeated observations on a large number of cells under various experimental conditions. But even when this is done, it is still found that many central neurons are incapable of responding to electrophoretically administered catecholamines.

Hence, it seems reasonable at this time to assume that the property of responsiveness to catecholamines is linked somehow to certain cellular characteristics which are not common to all central neurons but are possessed only by some cells. The presence of adrenergic synapses could account for these characteristics, but alternative interpretations are also possible.

DIRECTION OF THE RESPONSE

NE has been shown to produce either of two effects on sensitive neurons, facilitation or depression of their activity. The latter effect is the most common, but in certain regions (*e.g.*, medulla) the opposite is true (9, 10). Facilitatory and inhibitory effects by NE have been observed also in the spinal cord (53, 61), hypothalamus (6), caudate nucleus (5) and in the olfactory bulb of anesthetized rabbits (60). In the latter regions, however, only depressant effects are observable in unanesthetized preparations (4, 50, 60). Similarly, depression is the rule for NE responses of pyramidal tract and hippocampal pyramidal layer neurons (57). On the other hand, Deiters nucleus neurons in the medulla are consistently facilitated by NE (63). Renshaw cells are almost invariably depressed by NE, but there are some in which an initial depression is followed by facilitation (61). Dopamine mainly depresses and rarely facilitates caudate nucleus neurons (5); it has also been reported to depress units in the cortex (37), the lateral geniculate (19) and the ventrobasal complex of the thalamus (2). E was found to depress cortical neurons when given in small doses but to have the opposite effect when given in larger doses (37).

It should be noted that qualitatively similar observations have been made for acetylcholine and serotonin responses of central mammalian neurons (48, 53). These compounds also can produce either facilitation or depression of central neurons when administered electrophoretically. Responses to acetylcholine also are consistently in one direction or the other among a population of nerve cells of the same functional type (*e.g.*, depression of mitral cells but facilitation of Renshaw cells, pyramidal tract, hippocampal, pyramidal, and Deiters nucleus neurons); depression preceding facilitation is a characteristic response to acetylcholine of pyramidal tract neurons (57) and a frequent response of units in the ventrobasal complex of the thalamus (1, 2). It should be noted, moreover, that spike inactivation or stabilization of the cell membrane do not account for depression of activity induced by NE or by acetylcholine or serotonin, since units made silent by administration of these drugs can still respond to electrical or chemical stimuli.

Taken together, these observations suggest that both facilitatory and depressant effects elicited by the same suspected transmitter are genuine phenomena, the direction of the response depending upon certain characteristics of the tested neuron. One possibility might be that there are two types of receptor for the same substance, one leading to conductance changes resulting in hyperpolarization of the cell membrane and depression of unit activity while the other would have the opposite effect, *i.e.*, depolarization of the cell membrane and facilitation of unit discharge. Some cells may possess only one type of receptor; others may have

both. In the latter case, the direction and the magnitude of the response would be determined by the ratio of the two receptors and by their relative threshold and accessibility.

Hence, leaving aside the question of significance relative to synaptic transmission, facilitation or depression by the same substance may well reflect the predominant receptor type present on the cell membrane and accessible to the drug. However, alternative mechanisms must be considered. For example, either facilitation or depression could result 1) from chemical or functional competition with an unknown transmitter, 2) from specific or nonspecific effects on axon terminals causing or preventing transmitter release, 3) from actions upon neighboring units functionally related to the neuron under observation, *etc.* Tests to distinguish among these various potential sites of action can be devised (54) but have not yet been systematically applied.

OTHER FEATURES OF THE RESPONSE

Irrespective of their direction, responses of central neurons to NE are of surprisingly long duration. For a 5- to 30-sec period of application, responses may last for up to 5 min, although more typically 1 to 2 min. Responses commonly show also a delayed onset, on the order of 5 to 30 sec from the start of drug administration. Quickly responding units also have been observed (6) but they represent the exception rather than the rule; in any event, the latency of their response to NE, between 1 and 2 sec, is much greater than that exhibited by Renshaw cells in response to eletrophoretically administered acetylcholine, in the order of 0.03 to 1.0 sec (20, 21). Like most other central neurons, however, Renshaw cells show a delayed and prolonged response to NE (53, 61).

Many factors can contribute to individual differences in the latency of onset and in the duration of the responses of central neurons to a drug. When technical factors (17, 35) related to the methodology of drug administration and testing are excluded, several biological factors must be considered (53). These include the size of the neuron, the location and relative concentration of membrane receptive sites accessible to the drug and their distance from the site of drug administration, the types and the efficiency of local diffusional and enzymatic barriers, the presence or absence of specific or nonspecific binding sites on the path of the drug molecules, the association and dissociation constants of drug-receptor interactions, the presence or absence of receptive sites of different threshold and opposite function on the same cell, the functional relationship of the tested unit *vis a vis* other nerve cells in a path of self-sustained reverberating activity, the activation or inactivation of functionally related neighboring units, *etc.* It is clearly impossible to assess the exact contribution made by each of these factors in any particular case and it can be assumed that individual variations in the speed, direction, pattern, and duration of the response reflect in large measure the individual characteristics of of the tested neuron.

Nevertheless, the finding that, in spite of individual variations, most central neurons yield delayed and prolonged responses to NE is puzzling. A delayed response could be expected if the drug were to produce its observed effect in-

directly, through actions on neighboring units, reflexly affecting the recorded neuron through mono- or polysynaptic pathways. Similarly, a prolonged effect could result if the activity of the affected neuron were critical in the maintenance of activity in a reverberating circuit. While mechanisms of this type may be operative in some cases, it is highly improbable that they would be operative in all. It is difficult to believe, in fact, that among the many thousand central neurons of different size, shape, locale and connections that have been tested with NE, indirect mediation of drug effect should be the rule rather than the exception.

If it were assumed as probable that central neurons responses to electrophoretically administered NE often reflect a direct drug action on the affected cell, it becomes informative to compare the time course of the cell's response to NE with that of its response to a relatively nonspecific chemical stimulus, *e.g.*, L-glutamate (13, 21, 23), applied from the same electrode. Invariably, the latency of onset for responses to L-glutamate is shorter than for NE and the same is true for the duration of the cell response to the two drugs.

Differences in ion mobility or in the efficiency of physical and enzymatic barriers for NE and L-glutamate, respectively, may partially account for the different time course of cells responses to the two substances. It is also conceivable that a delayed onset could result from a relative paucity of NE receptors on central neurons or that both the delayed onset and the long duration of NE responses could be due to an inherent sluggishness of NE receptors (cf. Burnstock and Holman, Section V C).

Another explanation might be that the sites of predominant drug action may be different for NE and L-glutamate. Assuming the effects of L-glutamate to be nonspecific, it may be supposed that there will be plenty of "receptors" available on all portions of the cell membrane and that a response will be elicited as soon as drug molecules will have reached the nearest portion of the membrane. With NE and other suspected transmitters, on the other hand, the drug molecules must first find the "right" receptive sites, wherever these may be in relation to the point of drug administration. The latter is likely to be nearer the perikaryon than the dendrites of the recorded unit since drugs are usually administered at points where the extracellularly recorded action potentials are of relatively maximal size, presumably obtainable when the microelectrode tip is near the source of the action potentials, *i.e.*, the cell body. Hence, when technical factors are excluded, a short-latency response to a suspected transmitter may imply that appropriate receptors are within easy reach, as would be the case if many of these receptors were on the cell body. In this case, moreover, only minor differences would be expected in the latencies of response to the suspected transmitter and to a nonspecific chemical stimulant, as observed for Renshaw cells responses to acetylcholine and L-glutamate respectively (*cf.* 1, 21, 23). A delayed response, on the other hand, would imply that most of the receptors are farther away, perhaps on dendrites, needing to be recruited before an effect can be detected.

A similar argument could be advanced to explain the long time course of the response. Assuming a somatic site of action of NE and of the other suspected transmitters, their effect should cease soon after their administration is dis-

continued, unless it were postulated that there is only minimal or no active mechanism for their removal and that their dissociation constants are much longer than would be expected from transmitters acting on the soma, as may be inferred from the available data on the duration of postsynaptic potentials (30). Actions at dendritic sites, on the other hand, may cause subtle excitability changes which could well be of long duration.

While frankly speculative, this line of argument leads to the conclusion that if NE is a central transmitter, there should be a wealth of adrenergic synapses on dendrites, and the same should hold true for synapses operated by acetylcholine and serotonin, with the exception of Renshaw cells, where cholinergic synapses should be mainly on the cell body.

A POSSIBLE CENTRAL ADRENERGIC SYNAPSE

The only central synapse for which there is now relatively convincing pharmacological evidence that it may be adrenergic is part of an inhibitory pathway in the rabbit olfactory bulb (50). Within this structure, nerve cells are arranged in concentric layers, and the bodies of several cell types (glomerular, tufted, mitral, and granule) are largely restricted to different layers. The axons of mitral cells form the lateral olfactory tract (LOT), which ends mainly in the prepyriform cortex. Electrical stimulation of the LOT brings about a relatively prolonged (40 to 150 msec) inhibition of the spontaneous firing of ipsilateral mitral cells (hereafter called LOT inhibitory response), which is accompanied by hyperpolarization of the cell membrane (43, 64) and is commonly thought to result from activation of recurrent axon-collaterals of mitral cells (32, 55, 64). Nevertheless, centrifugal fibers (*i.e.*, afferents to the bulb from other central structures) are also present in the LOT (44); as will be seen, activation of some of these fibers by electrical stimulation of the LOT contributes to the duration of the LOT inhibitory response.

In a preliminary investigation (60), only some mitral cells and other rabbit olfactory bulb neurons were found to be responsive to the electrophoretic administration of acetylcholine, NE, and serotonin, but in subsequent studies (4, 50), presumably on account of improved techniques, it was found that a large proportion of mitral cells are sensitive to these substances. In unanesthetized rabbits, responses invariably consist of a depression of spontaneous unit activity. This finding and the biochemical evidence for the presence of acetylcholine, NE, and serotonin in the rabbit brain (8, 34, 59) suggested that one of these substances may mediate the LOT inhibitory responses.

It was found (50) that the electrophoretic administration to mitral cells of the acetylcholine antagonists atropine, hexamethonium, chlorisondamine (Ecolid), and dihydro-β-erythroidine blocked the cell's responsiveness to acetylcholine but did not significantly affect the duration of the LOT inhibitory response. Electrophoretic administration of N-(2-chloroethyl)-dibenzylamine hydrochloride (Dibenamine) was ineffective against responses to acetylcholine and serotonin but frequently reduced or blocked mitral cells responses to NE. When this occurred, the LOT inhibitory response was considerably shortened, although never

completely abolished. Similar effects were produced by phentolamine (Regitine), whereas dichloroisoproterenol was ineffective. LOT inhibitory responses were dramatically reduced by the electrophoretic administration of lysergic acid diethylamide (LSD-25) and its brom derivative (BOL-148) but these two substances proved to be more effective antagonists of NE than of serotonin in the rabbit olfactory bulb. Intravenous reserpine reduced the duration of the LOT inhibitory response, leaving unchanged the response elicited by the electrophoretic administration of NE. Moreover, rabbits pretreated with α-methyl-*m*-tyrosine and tested 7 to 11 days later (at a time when NE stores are still reduced but those of serotonin and dopamine are backed to normal levels (12)), showed much shorter LOT inhibitory responses than those yielded by mitral cells in untreated animals under identical testing conditions. Finally, biochemical analysis of the olfactory bulb in untreated rabbits showed NE to be present in concentrations of 0.08 μg per g by the trihydroxyinole method, while epinephrine and dopamine were not detectable (62).

Since LOT inhibitory responses are still present (32) after degeneration of the centrifugal fibers that are present in the LOT together with the mitral cells' axons, it is probable that a portion of the LOT inhibitory responses is mediated by axon-collaterals of mitral cells (32, 55, 64) *via* direct inhibitory synapses with ipsilateral mitral cells (32), or *via* interneurons which in turn would inhibit mitral cells (55, 64) or, conceivably, *via* presynaptic inhibitory effects. However, histochemical observations presented during this symposium (27a) show that a portion of the centrifugal fibers present in the LOT contain NE and that these fibers end mainly in the granule layer. This finding, coupled with the pharmacological observations summarized above, suggests that a component of the LOT inhibitory response results indirectly through activation of these NE-containing fibers. Whether the latter cause their effects on mitral cells by excitation of interneurons which in turn inhibit mitral cells, or by inhibition of interneurons which tonically facilitate mitral cells, cannot be specified at the present time. Activity of granule cells is particularly difficult to record with 5-barreled micropipette electrodes (50), presumably because the action potentials produced by these cells are of such small amplitude, when recorded extracellularly, as to be buried in the base line noise. Hence, it is quite possible (50) that the depressant effect of NE on mitral cells may arise indirectly, by NE actions (excitatory or depressant) on a neighboring interneuron, reflexly affecting the mitral cell under observation. Be this as it may, the pharmacological and histochemical evidence now available leaves little doubt about the presence of an adrenergic synapse in the rabbit olfactory bulb.

SUMMARY

Studies by electrophoretic administration of NE directly at the site of extracellular unit recording have shown the presence of NE-sensitive units in all regions of the mammalian central nervous system thus far explored. These include the lumbar segments of the spinal cord, the medulla, the hypothalamus, the thalamus, the hippocampus, the red and caudate nuclei, the septum, and the

cortex in the cat, and the olfactory bulb in the rabbit. Most responsive cells are depressed by NE but others are consistently facilitated. Irrespective of their direction, NE responses have a long latency of onset and a long duration; these features could result from dendritic sites of drug action. There is evidence that NE is a synaptic transmitter in an inhibitory pathway in the rabbit olfactory bulb.

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