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Microiontophoretic studies of the effects of false transmitter candidates and amphetamine on cerebellar Purkinje cells

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The effects of microiontophoretic applications of equivalent doses (ejection times and currents) of noradrenaline, amphetamine, octopamine and *p*-hydroxynorephedrine on the spontaneous firing of Purkinje and unidentified cells in the cerebellum of rats were examined. In addition, the effects of amphetamine on Purkinje cells were examined in animals pretreated with the tyrosine hydroxylase inhibitor, α-methyltyrosine (α-MpT) or with a combination of reserpine plus α-MpT. The results indicate that the "false transmitters" are weak agonists when compared to noradrenaline in inhibiting the firing of Purkinje cells. The results of the iontophoretic studies with amphetamine are not consistent with a pre-synaptic releasing effect by amphetamine at noradrenergic synapses in the cns since the efficacy of amphetamine on Purkinje cells was unaltered after pretreatment with α-MpT or α-MpT plus reserpine.

The presence, accumulation and release of "false transmitters" or "cotransmitters" from noradrenergic neurons are of increasing interest in elucidating both the pharmacology and physiology of aminergic neuronal systems. In this regard, *p*-hydroxyphenylethanolamine (octopamine, OCT) has a wide species distribution and is present in mammalian cns (Molinoff & Axelrod, 1972). The substance accumulates in peripheral adrenergic neurons (Kakimoto & Armstrong, 1962) and various regions of the cns of rats (Boulton & Wu, 1971) following monoamine oxidase inhibition, the accumulated amine can be released by stimulation of adrenergic nerves (Kopin, 1968). *p*-Hydroxynorephedrine (PHN), a metabolite of amphetamine in some species, is found in heart (Goldstein & Anagnoste, 1965), spleen (Thoenen, Huerlimann & others, 1966) and brain (Groppetti & Costa, 1969; Brodie, Cho & Gessa, 1970; Lewander, 1970) following amphetamine administration and may act as a false transmitter in adrenergic neurons (Brodie & others, 1970). This possibility has received support by the demonstration that the localization and elimination of PHN resembles that of noradrenaline in the brains of rats (El Guedri, Jacquot & others, 1973).

With few exceptions, false transmitter candidates have not been tested using single unit recording and iontophoretic techniques for their ability to alter neuronal activity. Therefore, we have compared the effects of PHN and OCT with those of noradrenaline and amphetamine on the firing of Purkinje and unidentified cells in the cortex of the cerebellar vermis of rats. Purkinje cell responses were judged to be of particular interest in light of the elegant studies of Hoffer, Siggins & others (1973) postulating the existence of noradrenergic synapses on these cells. In addition, we have examined the effects of iontophoretically applied noradrenaline and amphetamine on Purkinje cells in animals pretreated with drugs which would be expected to attenuate the behavioural effects of systemically administered amphetamine (Stolk & Rech, 1970).

METHODS

Microiontophoresis

Male, Sprague-Dawley rats (175–250 g) were used. The animals were anaesthetized with either a mixture of O_2, N_2O and methoxy-flurane or chloral hydrate (350 mg kg^{-1}, i.p., initially with supplements as required) and body temperature was maintained at 37° via a servo device. Standard iontophoretic and extracellular recording techniques for measuring activity changes in single cells were used (Jordan & Phillis, 1972). The centre barrel of 6-barrelled micropipettes (tip diameters of 6–10 μm) was used for recording and was filled with 2M NaCl by centrifugation while the outer barrels were filled by centrifugation with the following solutions: (−)-noradrenaline HCl (0·2M, pH 4·2), (±)-octopamine HCl (0·2 M, pH 4·2), (+)-amphetamine sulphate (0·2 M, pH 4·3), *p*-hydroxynorephedrine HCl (0·2 M, pH 4·4) and 2M NaCl. The technique of current balancing at the electrode tip (Salmoiraghi & Weight, 1967) was used to reduce current artifacts and a cathodal current of 8–10 nA was used to retain the drugs in the micropipette between applications. A small burr hole was drilled through the calvarium overlying the cerebellar vermis and after the dura was opened, agar in 0·9% NaCl solution was used to prevent drying. Purkinje cells were identified by their characteristic irregular, high rate of spontaneous discharge and the presence of "inactivation" responses (Granit & Phillips, 1956; Woodward, Hoffer & Lapham, 1969). The unidentified cells were all spontaneously active.

Drug pretreatments and noradrenaline assay

Four animals were pretreated 2–5 h before recording with 200 mg kg^{-1} of α-methyl-*p*-tyrosine methylester HCl (α-MpT, administered i.p., dose calculated as free base). An additional four rats were administered 100 mg kg^{-1} of α-MpT 20 h (n=2) and 44 h (n=2) after an injection of 5 mg kg^{-1} of reserpine. Recordings from Purkinje cells were made 1–3 h after the last injection in these animals. To determine if the pretreatments were producing their predicted effects on noradrenergic mechanisms, the pretreated animals were killed immediately subsequent to the iontophoretic studies and the endogenous cerebral content of noradrenaline was determined fluorometrically according to a combination of the procedure described by Whitby, Axelrod & Weil-Malherbe (1961) and Chang (1964). The values obtained were compared to those from unanaesthetized control animals assayed simultaneously, which have been determined to be not significantly different from the values observed in operated, anaesthetized animals (unpublished observations).

RESULTS

False transmitters

OCT and PHN were weak agonists compared to noradrenaline (and amphetamine) in their abilities to inhibit cerebellar Purkinje cell firing. Using ejection times and currents equal to noradrenaline, OCT inhibited 10 out of 21 Purkinje cells depressed by noradrenaline and 9 of 17 unidentified cells in normal animals. Similarly, PHN inhibited 7 of 21 Purkinje cells and 8 of 17 unidentified cells. Furthermore, when OCT and PHN did inhibit cell firing, their effects were usually weak by comparison with noradrenaline or amphetamine, as illustrated in Fig. 1. Fig. 1B represents the firing of a Purkinje cell in an α-MpT treated animal showing potent inhibition by noradrenaline and amphetamine and weak inhibition during OCT and PHN application. Neither PHN nor OCT affected cells that were not inhibited by noradrenaline. While it was not possible to demonstrate a blockade of noradrenaline depression by

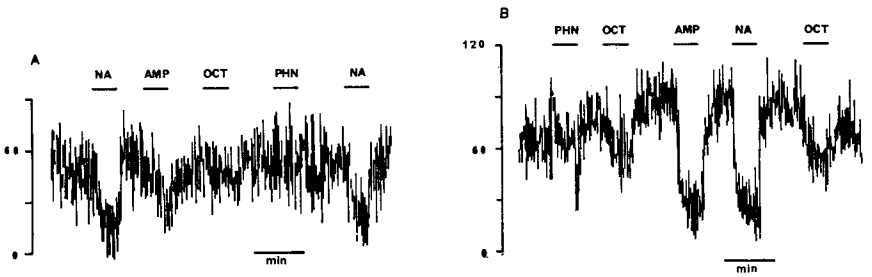

FIG. 1. A. Purkinje cell responses in a normal animal to iontophoretic applications (50 nA, 30 s) of NA (noradrenaline), AMP (amphetamine), OCT and PHN. The ordinate in this and other figures indicates the rate of cell firing in action potentials s^{-1}. The lines above the trace indicate periods of drug application.
B. Purkinje cell responses in an animal pretreated 3·5 h previously with 200 mg kg^{-1} of α-MpT to iontophoretic applications (100 nA, 30 s) of PHN, OCT, AMP and NA.

prior or concomitant application of the false transmitter candidates to Purkinje cells (using up to 100 nA's of current for up to 5 min periods), an additive effect of simultaneous applictions of noradrenaline and OCT or PHN was frequently observed. An example of this is shown in Fig. 2 where it can be seen that applications of normally ineffective doses of noradrenaline results in a depression (resembling that of a higher dose of noradrenaline) during concomitant iontophoresis of PHN or OCT.

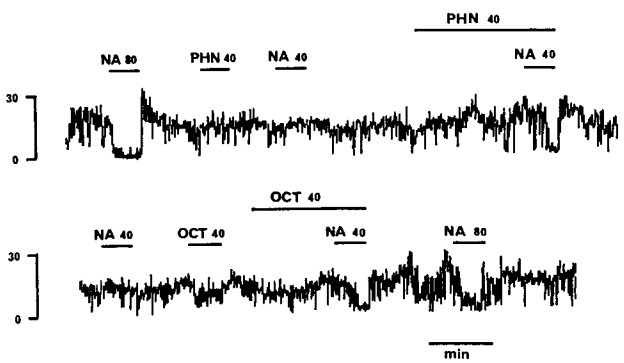

FIG. 2. Additive effect of PHN and OCT with NA to inhibit the firing of a Purkinje cell. The trace is continuous from top to bottom. The numbers above the trace indicate the amount of current (nA) used to eject the drugs.

Amphetamine studies

Iontophoretic applications of noradrenaline (20–100 nA, 30–60 s) depressed the firing rate of 21 out of 22 Purkinje cells and 17 out of 23 unidentified cells. Amphetamine, with ejection times and currents equal to noradrenaline, also inhibited the firing of a high percentage of both types of cells. On unidentified cells, amphetamine exhibited a depressant action in 15 out of 17 trials and failed to affect cells not inhibited by noradrenaline. As shown in Table 1, on Purkinje cells in naive animals, amphetamine inhibited the firing of 20 out of the 21 cells that were inhibited by noradrenaline. Only one Purkinje cell was found that was inhibited by AMP but not by a comparable dose of noradrenaline. Also summarized in Table 1 are the effects of noradrenaline and amphetamine on Purkinje cells in the animals pretreated with α-MpT or a combination of reserpine plus α-MpT. As can be seen, the effects of amphetamine were unaffected by these pretreatments. There was a 52% reduction

Table 1. *Failure of α-MpT or reserpine plus α-MpT pretreatment to influence amphetamine effects on Purkinje cells.* Equal doses (20–100 nA, 30–60 s) of noradrenaline and amphetamine were tested on each cell. The figures represent the number of neurons showing inhibition (—) or no effect (0).

Pretreatment as described in Methods.		Amphetamine	
		—	0
None	—	20	1
	Noradrenaline 0	0	1
		Amphetamine	
		—	0
α-MpT	—	12	0
	Noradrenaline 0	0	0
		Amphetamine	
		—	0
Reserpine + α-MpT	—	22	0
	Noradrenaline 0	2	1

in the cerebral content of noradrenaline in the α-MpT pretreated animals (0·20 μg g^{-1} with s.d. 0·05, n=4 *vs* 0·41 μg g^{-1} with s.d. 0·04, n=5) and an 88% reduction in the animals treated with reserpine plus α-MpT (0·06 μg g^{-1} with s.d. 0·02, n = 4 *vs* 0·51 μg g^{-1} s.d. 0·09, n = 4).

DISCUSSION

The assumption, on which our observations are based, is that the Purkinje cell of the cerebellar cortex constitutes an example of an identified noradrenergically innervated neuron (Hoffer & others 1973). Thus, agents applied in the vicinity of a Purkinje cell should have access to both post-synaptic noradrenergic receptors as well as pre-synaptic noradrenergic nerve terminals. Also, by testing substances before and after impairment of normal noradrenergic mechanisms in these terminals it should be possible to at least partially define pre- and post-synaptic actions of a drug.

The present experiments show that the action of amphetamine on Purkinje cells was unaltered by pretreatment with doses of α-MpT or reserpine plus α-MpT which were sufficient to substantially deplete cerebral noradrenaline. Our results are consistent with a direct post-synaptic action of amphetamine, in agreement with the conclusions of Feltz & de Champlain (1972) who observed no alterations in the inhibitory effects of amphetamine on caudate neurons after destruction of the pre-synaptic dopaminergic terminals with 6-hydroxydopamine or pre-treatment with reserpine plus α-MpT. Likewise, Hoffer, Siggins & Bloom (1971) have observed that amphetamine still elicited an inhibitory effect on Purkinje cell discharges in rats pretreated with 6-hydroxydopamine. On the other hand, Boakes, Bradley & Candy (1972) have reported that pretreatment with either 5 mg kg^{-1} of reserpine or 500 mg kg^{-1} of α-MpT dramatically reduced the ability of iontophoretically applied amphetamine to mimic (presumably through a pre-synaptic noradrenaline releasing mechanism) noradrenaline effects on brain stem neurons.

Thus, the hypothesis that amphetamine acts at central catecholamine synapses by releasing catecholamines from an α-MpT sensitive presynaptic pool, as has been inferred from behavioural and biochemical studies (see Sulser & Sanders-Bush, 1971),

finds support at the single-cell level in the experiments on unidentified brain stem, neurons (Boakes & others, 1972) but it is difficult to reconcile with our results and those of Hoffer & others (1971) on Purkinje cells and the findings of Feltz & de Champlain (1972) on caudate neurons. Whether or not this discrepancy represents a genuine regional difference in the actions of amphetamine or is due to other considerations remains unknown. It is conceivable that a small "functional pool" of noradrenaline was left intact by our pretreatment regimen or that tyrosine hydroxylase activity was not entirely inhibited throughout the time course of our iontophoretic experiments. However, these possibilities do not easily accord with our failure to discern any changes at all in the efficacy of amphetamine either during or throughout the iontophoretic experiments.

With regard to our results with OCT and PHN, while it is not implicit in the concept of "false transmitters", it might be predicted that candidates for such a role in the cns might possess an efficacy in affecting neuronal activity different from the normal or primary transmitter. If OCT and PHN do act as false transmitters in central noradrenergic nerves, then the results of our studies appear to support such a prediction with regard to the effects of these substances on noradrenergically innervated neurons in the cerebellum. Thus, while affecting cells that were inhibited by noradrenaline by comparison with noradrenaline they produced rather slight inhibitions of Purkinje cell firing.

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