EXCITATORY EFFECTS OF DOPAMINE ON MOLLUSCAN NEURONES

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DOPAMINE (DA) is the only catecholamine which has been detected in appreciable amounts in the nervous system of Molluscs (SWEENEY, 1963; see GERSCHENFELD, 1973, for a review). It is particularly abundant in certain ganglia, and can sometimes be localised in identifiable neurones (MARSDEN and KERKUT, 1970). The demonstration that DA is a transmitter in the molluscan nervous system is, however, still incomplete. Liberation of DA by stimulation of the ganglia has been observed, but in experimental conditions where one could fear serious—and still unexplained—artefacts (ASCHER, GLOWINSKI, TAUC and TAXI, 1968). Application of DA on molluscan neurones has shown that it could trigger a variety of effects (see GERSCHENFELD, 1973) but, primarily because of an incomplete knowledge of the pharmacological properties and of the ionic mechanisms of DA responses, no identifiable synaptic potential could be securely claimed to be due to DA, until very recently (BERRY and COTTRELL, 1973).

In the experiments to be described, an attempt was made to characterise the responses of identified *Aplysia* neurones to electrophoretic applications of DA. Special emphasis will be placed on the excitatory effects of DA, since they have been only rarely observed elsewhere. The methods used in these experiments have been described in a previous article (ASCHER, 1972).

DA EFFECTS CAN BE EXCITATORY, INHIBITORY, OR EXCITATORY-INHIBITORY

The effects of DA differ according to the cell studied. For example, in the pleural ganglia of *Aplysia*, the "anterior" cells are excited by DA while the "medial" cells are inhibited, and most "posterior" cells show a biphasic, excitatory-inhibitory response to locally applied DA.

Most previous studies of DA effects, even in Molluscs, reported mainly or exclusively inhibitory effects. The frequent observation of excitatory effects of DA in these studies on *Aplysia* can probably be ascribed to differences in experimental conditions.

First, it appears that DA excitatory effects are by far more prone to desensitisation than inhibitory responses. For example, in cells presenting a biphasic response to a short (200 msec) electrophoretic pulse of DA, repetition of the injection at frequencies as low as 1/min often produces a progressive decrease of the excitatory component, while the inhibitory one is hardly affected. On these same cells, the response to a longer pulse will appear as a very prolonged inhibition following a short excitation, and DA applied in perfusion will show only the inhibitory component. It thus appears that the mode of application of DA is a critical requirement for the observation of DA excitatory effects.

But even when DA is applied with short electrophoretic pulses appropriately spaced, the occurrence of an excitatory component will depend critically on the positioning of the pipette. Considering again the posterior pleural cells of *Aplysia*,

it was observed that if the DA pipette was progressively withdrawn from a position where the injection elicited a biphasic response, the excitatory component was the first to disappear. This may explain that if the pipette is positioned "blindly", a variety of positions will be found from which the response will appear purely inhibitory.

PHARMACOLOGICAL PROPERTIES OF DA EXCITATORY RESPONSES

Various antagonists were applied to the preparation in an attempt to demonstrate, in a purely qualitative way, that DA receptors mediating excitatory responses were (1) different from the DA receptors mediating inhibitory responses and (2) different from the acetyl-choline (ACh) receptors mediating excitatory responses.

For the first purpose, the most convenient cells were again the posterior cells of the pleural ganglia, on which DA produces a biphasic response. It was observed that ergometrine (WALKER, WOODRUFF, GLAIZNER, SEDDEN and KERKUT, 1968) and methyl-ergometrine produced a selective elimination of the inhibitory component, whereas curare and strychnine selectively eliminated the excitatory component. These pharmacological differences were confirmed in other cells, and in particular in cells of the visceral ganglion where DA usually elicits only inhibitory responses, but where an excitatory component could be unmasked after application of methyl-ergometrine.

Although these data show that different receptors mediate the excitatory and inhibitory DA responses, they did not discriminate between the receptors mediating DA and ACh excitatory effects. These however, could be also differentiated. On the anterior pleural cells, on which both DA and ACh elicit predominantly excitatory effects, hexamethonium blocked selectively the ACh response, whereas DA in perfusion abolished selectively the DA response.

The possible use of the above data is exemplified by the recent observations of BERRY and COTTRELL (1973) in the pleural ganglion of *Planorbis*. Stimulation of giant neuron presumed to contain DA triggered, in various "post-synaptic" cells, synaptic potentials which were excitatory or excitatory-inhibitory. The pharmacological properties of these synaptic potentials resemble those of the DA responses, thus strengthening the case for the presence of dopaminergic synapses in molluscan ganglia.

COMPARISON OF THE IONIC MECHANISMS INVOLVED IN DA AND ACh EXCITATORY EFFECTS

The electrophysiological characterisation of the ionic permeability changes underlying a transmitter action usually requires the measurement of an "inversion potential" in various ionic environments. In the case of DA effects on *Aplysia* neurones, this could be done for many *inhibitory* responses, which were shown to be due to an increased K^+ permeability (ASCHER, 1972). But attempts to measure an inversion potential for *excitatory* DA responses were not as successful. Delayed rectification, which causes a dramatic fall in the cell's input resistance, precluded the observation of responses on strongly depolarised neurones, and thus impeded the direct measurement of an inversion potential. Anomalous rectification (KANDEL and TAUC, 1966), on the other hand, interfered with the estimation of an inversion potential by extrapolation from the responses studied on hyperpolarised neurons.

This last difficulty could sometimes be overcome by manipulation of the external

K^+ concentration, or by addition of Cs^+ to the sea water. The first series of records in Fig. 1 show that in normal sea water, bringing the membrane potential from −60 to −90 mV caused a decrease in membrane resistance, no change in the amplitude of the ACh response, and a reduction in the amplitude of the DA response. After reducing the external K^+ concentration, however, the reduction of input resistance was no longer observed when going from −60 to −90 mV, and in these conditions both the DA and ACh responses increased with hyperpolarisation of the membrane.

These findings indicate that both the DA and the ACh responses result from an increase in membrane conductance. In addition, the figure shows that the effect of polarisation is more marked on the ACh response, which indicates a more negative value of the (extrapolated) inversion potential. Indeed for ACh responses an inversion potential could sometimes be directly measured at membrane potentials between 0 and −30 mV. Further studies showed, however, that these cases corresponded to ACh responses resulting from the superposition of two effects : the "true" excitatory effect of ACh—defined by its sensitivity to hexamethonium (TAUC and GERSCHENFELD, 1962)—and an hexamethonium-resistant increase in chloride conductance. This latter conductance change pulls the "global" inversion potential towards the chloride equilibrium potential, and accounts for the sensitivity of the ACh response to changes in chloride concentrations (ASCHER, GERSCHENFELD and KEHOE,1972).

A similar problem was encountered when an extrapolated value of the inversion potential was calculated for DA responses. The results showed an important scatter (between +30 and −10 mV), most of which is probably due to the superposition of "true" excitatory DA effects (defined by their curare sensitivity) and of inhibitory DA effects—the relative contribution of the two depending on the position of the pipette, the duration of the injection. . . (see above).

These complications prevented a full analysis of the ionic mechanisms of DA and

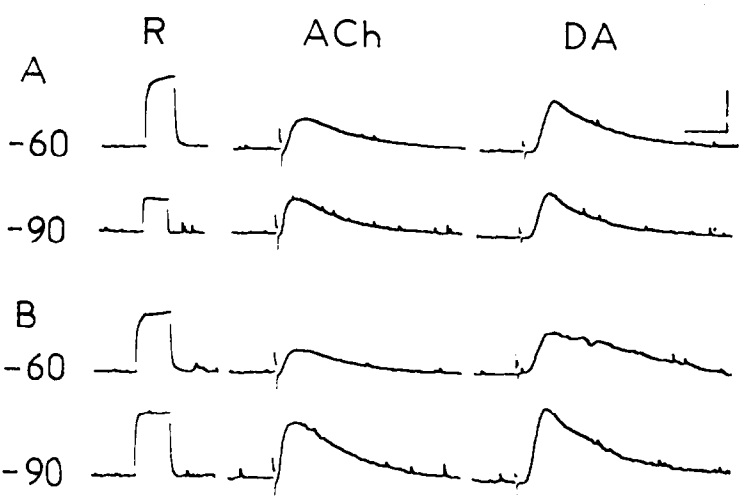

FIG. 1.—Effects of membrane polarisation on the amplitude of DA and ACh responses. Anterior pleural neurone. R: response to a square current pulse. ACh, DA: responses to electrophoretic applications of DA and ACh. The membrane potential was changed from −60 to −90 mV in normal sea-water (A) and in low K^+ (1 mM) sea-water (B). The suppression of anomalous rectification in (B) "normalised" the behavior of DA and ACh responses as a function of membrane polarisation. Calibration 4 sec, 5 mV.

ACh excitatory effects. However, these two types of effects were observed to react differently to various ionic substitutions in the sea water perfusing the ganglion, as shown by the examples illustrated in Fig. 2.

Replacement of Na^+ by Li^+ markedly reduced the DA response, but only slightly affected that of ACh. After returning to Na^+ sea-water, the ACh respose was temporarily increased, while the DA response remained depressed for many minutes.

The effects of Li^+ are difficult to interpret. In squid axon, Li^+ sea-water may lead

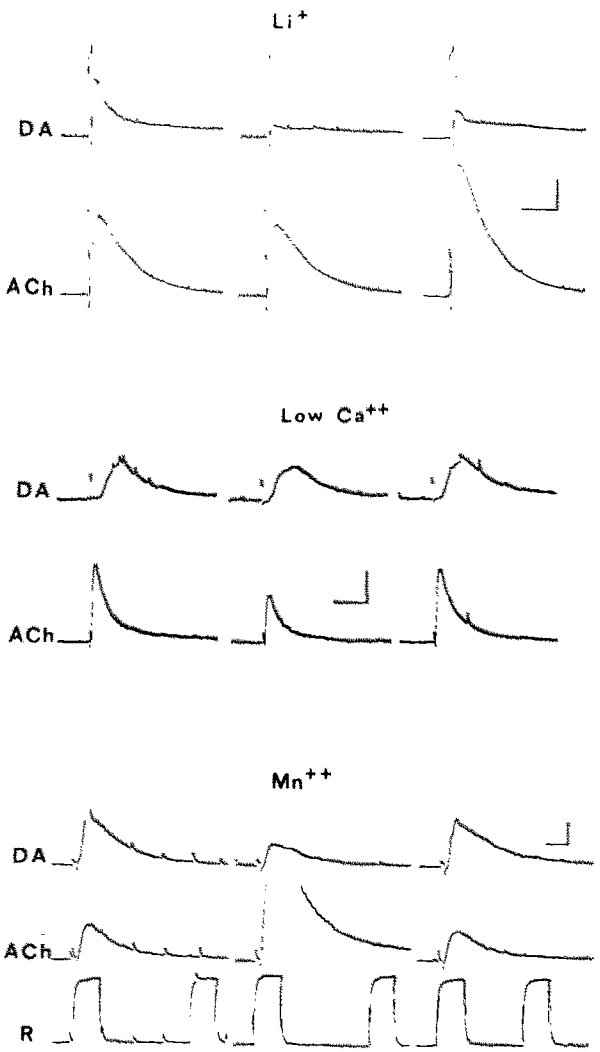

FIG. 2.—Effects of various ionic substitutions on DA and ACh excitatory responses. Anterior pleural neurone. R: response to a square current pulse. On each line the left record represents the initial response (in normal sea-water); the center one the response in modified sea-water (Li^+: after replacement of half external Na^+ by Li^+; low Ca^{2+}: after reduction of the external Ca^{2+} concentration from 10 to 0·3 mM; Mn^{2+}: after replacement of 50 mM/l $MgCl_2$ by 50 mM/l $MnCl_2$); the right record represents the response 3 min after returning to normal sea-water. Calibration 5mV; (Li^+): 10 sec; (Low Ca^{2+}): 4 sec; (Mn^{2+}): 1 sec.

to a reduction of the Ca^{2+} electrochemical gradient (BAKER, BLAUSTEIN, HODGKIN and STEINHARDT, 1969). However, in the case studied, reduction of the Ca^{2+} concentration did not mimick the effects of Li^{+} : on the contrary, it caused a reduction of the ACh response, but had little effect of the DA response (Fig. 2, Center).

It could tentatively be suggested that the DA excitatory action is due to a very selective increase of Na^{+} permeability (in which neither Li^{+} nor Ca^{2+} could replace Na^{+}), whereas the ACh excitatory action would correspond to the opening of a less specific channel. Such an interpretation is probably premature however. It does not account for the slow (and often only partial) recovery of the DA response after returning from Li^{+} to Na^{+} sea-water. It does not explain the complex effects observed when Mg^{2+} ions were substituted by various divalent ions—in particular Co^{2+} or Mn^{2+}: for example, as can be seen in Fig. 2, Mn^{2+} caused simultaneously a reduction of the DA response and a marked increase of the ACh response.

These results do not completely reveal the ionic mechanisms of DA and ACh responses. They do, however, offer additional clues for the identification of dopaminergic excitatory synaptic potentials.

REFERENCES

ASCHER P. (1972) *J. Physiol. Lond.* **225,** 173–209.
ASCHER P., GERSCHENFELD H. M. and KEHOE J. S. (1972) *J. de Physiol.* **65,** 92A.
ASCHER P., GLOWINSKI J., TAUC L. and TAXI J. (1968) *Adv. Pharmacol.* **6A**; 365–368.
BAKER P. F., BLAUSTEIN M. P., HODGKIN A. L. and STEINHARDT R. A. (1969) *J. Physiol.* **200,**431–458
BERRY M. S. and COTTRELL G. A. (1973) *Nature New Biol.* **242,** 250–253.
GERSCHENFELD H. M. (1973) *Physiol. Rev.* **53,** 1–119.
KANDEL E. R. and TAUC L. (1966) *J. Physiol.* **183,** 287–304.
MARSDEN C. and KERKUT G. A. (1970) *Comp. Gen. Pharmacol.* **1,** 101–116.
SWEENEY D. (1963) *Science N.Y.* **139,** 1051.
TAUC L. and GERSCHENFELD H. M. (1962) *J. Neurophysiol.* **25,** 236–262.
WALKER R. J., WOODRUFF G. N., GLAIZNER B., SEDDEN C. B. and KERKUT G. A. (1968) *Comp. Biochem. Physiol.* **24,** 455–470.