Short communications

Short-latency excitation of brain stem neurones in the rat by acetylcholine

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A fast excitatory response to acetylcholine (ACh) which has not previously been reported, has been found in the rat brain stem. Micro-iontophoretic applications of ACh to single brain stem neurones in unanaesthetized rats excited 81% and inhibited 3% of neurones studied. Two types of excitatory response were distinguished by their time course. Type I ACh excitation of neurones was of long latency resembling that previously reported in various parts of the brain. Type II excitation was of short latency, similar to that of micro-iontophoretically applied glutamate ions and to ACh excitation of Renshaw cells.

It has been suggested that the characteristics of the excitation produced by micro-iontophoretic application of acetylcholine (ACh) to single neurones in the brain are not those expected of an excitatory transmitter (Curtis & Koizumi, 1961 ; Krnjević, 1969) and that amino acids are more 'transmitter-like' in their rapidity of action and quick reversibility (Aprison & Werman, 1968). An excitatory transmitter role for ACh has been established in Renshaw cells in the spinal cord where Curtis & Eccles (1958) showed that excitation by micro-iontophoretically applied ACh occurred very rapidly and that the excitation was terminated very soon after the end of the application.

We wish to report the finding of a similar short-latency ACh excitation of single neurones in the rat brain stem.

Methods.—Adult rats were decerebrated under halothane anaesthesia (<0·5%) and the medial portion of the cerebellum was removed to expose the floor of the fourth ventricle. Anaesthesia was then discontinued.

Multibarrelled glass micropipettes were used to record neuronal activity and to eject ions from aqueous solutions of acetylcholine chloride (5–10%, pH 4·0–5·0, Hopkin & Williams or B.D.H. Ltd.) and monosodium glutamate (10%, pH 8·0–9·0, L. Light & Co.). Only spontaneously active neurones were studied.

Electrode penetrations were made between 1 and 4 mm rostral to the obex, between 1·5 mm either side of the midline and to a depth of 2·5 mm. Penetrations in the midline were avoided. The neuronal firing rate was electronically counted (Bradley & Wolstencroft, 1964) as the number of spikes occurring in epochs of 5 s duration. The time course of the responses was also determined from filmed records.

Drugs were usually ejected with a current of 50 nA for one or more 5 s periods. A retaining current of 15–40 nA prevented unwanted diffusion from the micropipettes. Previous studies in this laboratory (Bradley & Candy, 1970) have demonstrated a linear relationship between iontophoretic current and the quantity of ACh released.

Results.—Of 229 neurones studied, 184 (81%) responded with an increase, and 6 (3%) with a decrease in firing rate to iontophoretically applied ACh. Two types of excitation could be distinguished and were defined as those occurring within the first epoch after switching on the current (Type II excitation) and those which occurred during the second or subsequent epochs (Type I excitation). An increase in the neuronal firing rate of at least 50% over the mean spontaneous rate was taken as the criterion for excitation.

Figure 1A illustrates a neurone which was classified as exhibiting Type I excitation. The characteristics of this type of excitation, measured on the epoch basis, were that it occurred with a latency of 5–20 s after the start of the iontophoretic application and reached a maximum 10–20 s later. The firing rate returned to the original level 10–40 s after the current was switched off. Twenty-four percent of neurones excited by ACh were of this type.

The Type II response always occurred within the first 5 s epoch and reached a peak within 5–10 s of switching on the current (Fig. 1B). The response began to decay immediately after the ejecting current was switched off. Return to the original firing rate occurred some 5 s later. Seventy-six percent of neurones excited by ACh were Type II.

Filmed records of 30 neurones of each type were analysed to enable a more ac-

curate determination of the latencies of onset of excitation to be made. The criterion used to define latency in this case was that point at which the mean interspike interval was reduced by at least 50%. The latency of onset of most neurones already classified as Type I was greater than 5 seconds. However, the latency of onset of Type II excitation occurred within 0·2–0·8 s after the start of the application.

In view of the short latency of the Type II excitatory response, it was compared with that of glutamate ions, applied to the same neurone. The excitatory effect of glutamate occurred after a latency of 50–400 ms and the firing rate returned to its original level 1–5 s after switching off the ejecting current (Fig. 1C). The latency of onset of Type II ACh excitation did not appear to change when ACh was applied with currents of different strengths (0–80 nA). However, the magnitude and decay time of this response were dependent on the current strength as has been reported for ACh excitation in the cat brain stem (Bradley, Dhawan & Wolstencroft, 1966).

No correlation was observed between the spontaneous neuronal firing rate and latency of onset of ACh excitation, nor was there any correlation between the type of response and the individual electrodes or magnitude of backing current. Desensitization to the effects of ACh was rarely observed although this has been shown in other areas of the brain (McCance, Phillis & Westerman, 1968 ; Tebēcis, 1970).

Discussion.—Most cholinoceptive neurones in the rat brain stem were found to be excited by ACh (81%) and few were inhibited (3%), in contrast to previous studies (Schmidt, Krug, Maier, Pohle & Matthies, 1967 ; Couch, 1970). However, the use of gallamine-immobilized animals or barbiturate anaesthesia in the earlier studies might explain these differences. Pentobarbitone anaesthesia has been shown to reduce significantly the number of neurones excited by ACh in the rat brain stem (Dray, 1971, Bradley & Dray, 1972). The proportion of cholinoceptive neurones in the rat are also at variance with those reported in the cat by Bradley *et al.* (1966) who found 35.5% excited and 22% inhibited.

Two types of excitatory responses to iontophoretically applied ACh, clearly distinguishable by their respective time courses, have been observed. An excitatory response with a prolonged time course resembling that described for the cat brain stem (Bradley, *et al.*, 1966) was observed less frequently than the excitatory response

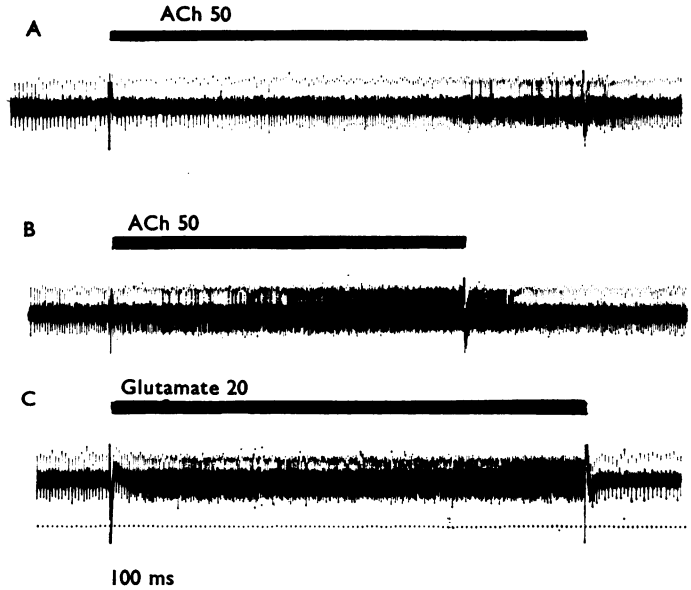

FIG. 1. Action potentials of brain stem neurones showing excitatory responses to microiontophoretically applied acetylcholine (ACh) and glutamate. Drug applications are indicated by the horizontal bars and current in nA is given above. A, Type I ACh excitation. B, Type II ACh excitation. C, Glutamate excitation of the same neurone as in B.

which occurred with a relatively short latency of onset and restricted time course.

Curtis & Eccles (1958) showed that micro-iontophoretically applied ACh could excite Renshaw cells with a latency of 30–1,000 ms. Since excitation by ACh of single neurones in other areas of the brain, especially the cerebral cortex, has been shown to occur with much longer latencies, it was suggested that ACh did not entirely fulfil the criteria expected of an excitatory transmitter (Krnjević, 1969).

In the present study it was found that excitation of single neurones in the rat brain stem by ACh occurred with a time course similar to that of an excitant amino acid and of ACh excitation of Renshaw cells. A fast response of this type to ACh has not previously been reported in the brain. Thus, the objections to a transmitter role for ACh based on its slowness of action after micro-iontophoretic application seems to be invalid, at least for neurones in the rat brain stem.

It is possible that the differences both in the time course of ACh excitation and the proportions of neurones responding to ACh in the brain stem of the rat and cat may be due to species differences.

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