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PROBLEMS ASSOCIATED WITH THE USE OF TETRAETHYLAMMONIUM TO TEST FOR MONOSYNAPTIC CONNEXIONS

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SUMMARY

Several investigators of the molluscan nervous system have used TEA, injected into presynaptic neurones, to determine whether the connexions made by these neurones are monosynaptic. The increase in spike duration produced by the TEA causes an increase in transmitter release, and hence an increase in the amplitude of the postsynaptic potential if the connexion is direct. If the connexion is indirect, the spike in an intercalated neurone will not be affected by the TEA, and the postsynaptic response will remain constant. Experiments described here show that TEA can cross electrotonic junctions in the gastropod mollusc *Planorbis corneus*. They also show that each TEA-prolonged presynaptic impulse may produce more than one postsynaptic impulse. A larger postsynaptic potential could therefore be produced by presynaptic injection of TEA in the case of an indirect connexion. This indicates that care must be taken when interpreting the results of experiments using TEA to test for monosynaptic connexions.

INTRODUCTION

Tetraethylammonium ions (TEA) have been used in certain invertebrate nervous systems to determine whether synaptic connexions between particular neurones are direct or indirect (Kehoe, 1969, 1972; Paupardin-Tritsch & Gerschenfeld, 1973; Cottrell & Macon, 1974; Gerschenfeld & Paupardin-Tritsch, 1974). Intracellularly injected TEA blocks the K^+-conductance increase associated with the action potential (Armstrong, 1969; Hille, 1967, 1970), thus prolonging its duration and causing an increase in transmitter release (Katz & Miledi, 1967; Kusano, Livengood & Werman, 1967). When injected into the presynaptic neurone the gradual increase in spike duration results in a related progressive increase in the amplitude and duration of the recorded postsynaptic potential, in the case of a direct connexion. If the connexion is indirect, the spike in an intercalated neurone will not be affected by the TEA, which is well retained within neurones, and the postsynaptic response will remain constant.

However, it was pointed out by Deschênes & Bennett (1974) that TEA may cross electrotonic junctions and thus lengthen the spike in an intercalated neurone coupled electrically to the injected presynaptic neurone. TEA is known to cross such

junctions in the vertebrate heart (Weingart, 1972, 1974), and Deschênes and Bennet showed that TEA crosses the septal junctions of crayfish giant axons. Since experiments using TEA to test for monosynaptic connexions appear to have been restricted to certain gastropod molluscs, we have investigated whether TEA can cross electrotonic synapses in the gastropod *Planorbis corneus*.

MATERIALS AND METHODS

Specimens of *Planorbis corneus*, obtained from Gerard & Haig Ltd, East Preston, Sussex, were maintained in aquaria at room temperature (15–22 °C).

Experiments were performed on the isolated buccal and circumoesophageal ganglia which were pinned to a plastic sheet at the base of a 10 ml Perspex chamber and bathed with continuously flowing physiological solution (Berry, 1972*a*) at room temperature. Double-barrelled microelectrodes containing 0·5 M-K_2SO_4 were used for intracellular recording and stimulation; their resistance ranged from 10 to 25 MΩ. For injection of TEA the K_2SO_4 in one barrel was replaced by 1 M TEA-bromide, and TEA ions were expelled from the microelectrode by direct current passed between the barrels. The current was not measured but could be adjusted to the desired value by periodic observation of the spike duration. Cells were impaled without first removing the overlying connective tissue. Records were made on a Brush 220 series two-channel ink recorder. For accurate measurement of rapid events, photographic records were made of the oscilloscope trace.

RESULTS

Experiments were performed on a symmetric pair of electrically coupled neurones in the buccal ganglia (Berry, 1972*b*). The perikarya, which are about 80 μm in diameter and about 900 μm apart, are situated on the dorsal surface of the ganglia close to the cerebro-buccal connectives (Fig. 1). Depolarizing and hyperpolarizing current pulses applied to either neurone are transmitted to the other, and spikes may be transmitted in both directions (Berry, 1972*b*).

TEA was injected into one of these neurones (designated presynaptic, Fig. 2A) while recording simultaneously from the other (postsynaptic, Fig. 2B). After 2–4 h injection (B2) there was an obvious increase in the duration of the postsynaptic spike, with further increase over the following 4 h (B3). These changes suggest that there was transfer of TEA across the synapse. Careful controls (Fig. 2C) showed that prolonged impalement of these neurones did not result in lengthening of the spike provided that there was no obvious damage, indicated by a reduction in spike amplitude. Postsynaptic spikes were elicited by just-threshold depolarization rather than presynaptic activation to prevent the broadening of the presynaptic spike from contributing to that of the postsynaptic.

TEA was also found to affect synaptic transmission between these cells; the prolonged presynaptic spike produced a larger postsynaptic (coupling) potential (Fig. 3A). In a few preparations where there was initially one-for-one transmission of action potentials, each TEA-prolonged presynaptic spike often produced two or three postsynaptic spikes (Fig. 3B).

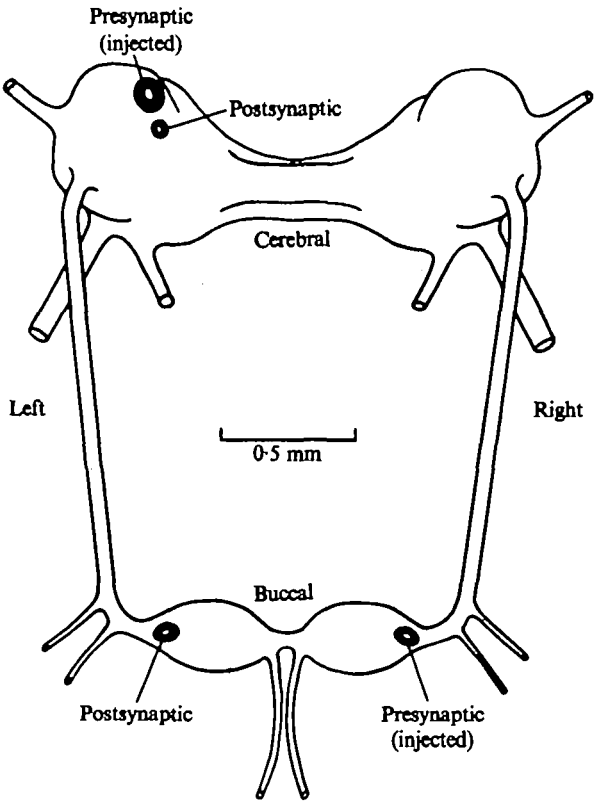

Fig. 1. Diagram of the dorsal surface of the buccal ganglia and the ventral surface of the cerebral ganglia, showing the positions of the electrically coupled neurones recorded in Figs. 2–4. The neurones in the cerebral ganglia are electrically coupled to at least six neighbouring neurones. The largest of these neurones (80–100 μm) is repeatedly identifiable.

Experiments were also performed on a group of electrically coupled neurones in the left cerebral ganglion, illustrated in Fig. 1. One of these neurones is identifiable because of its size and position, and this cell was injected with TEA while recording from nearby coupled cells. Many of the coupled cells produce small, short-duration spikes with a large undershoot (Fig. 4A). It appears that prolonged K^+-activation may start early in the rising phase of the spike to produce the observed shape. After 10 h injection the postsynaptic spike was slightly longer, and lacked the marked undershoot (Fig. 4B). This result is most easily explained by transfer of TEA and resultant decrease in K^+-activation. Control experiments showed that these changes did not occur in the absence of TEA injection.

DISCUSSION

The amounts of TEA injected in these experiments are larger than those used to test for monosynaptic connexions when judged by comparing the increase in spike duration. However, when using TEA to increase transmitter release, only a small increase in spike duration is necessary (Kehoe, 1969); the increase in post-synaptic response thus provides a very sensitive means of detecting TEA at the

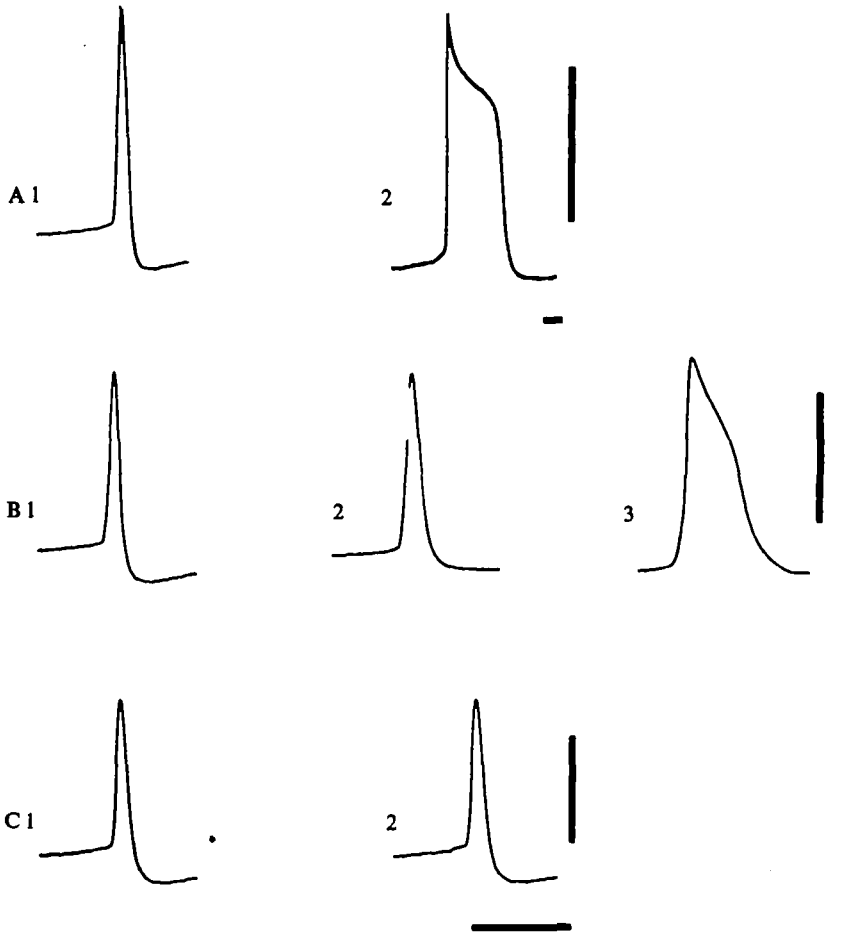

Fig. 2. Transfer of TEA between the electrically coupled neurones in the buccal ganglia. A 1, Presynaptic action potential (right neurone) before injection of TEA. A 2, Presynaptic action potential 7 h after the start of a 2 h period of TEA injection. Note the different time scale for this record. B 1, Postsynaptic action potential before presynaptic injection of TEA. B 2, Postsynaptic action potential 3 h after the start of presynaptic injection of TEA. Note the lengthening of the spike and reduction in its undershoot. B 3, Postsynaptic action potential 7 h after the start of presynaptic injection of TEA. The duration is considerably increased and the undershoot almost abolished. The microelectrode was removed from the cell between records. The results show the transfer of TEA between the neurones. C 1, Action potential recorded from one of the coupled neurones in a different preparation. C 2, Action potential recorded from the same neurone after 5 h continuous impalement with a double-barrelled electrode containing no TEA. On reimpalement of the neurone 24 h later, the amplitude and duration of the action potential were unchanged. Provided there was no reduction in spike amplitude, there was found to be little increase in spike duration or configuration after prolonged periods of impalement. This indicates that the postsynaptic responses to presynaptic TEA injection are a direct result of the TEA. Time scales: 100 msec; the scale for A 2 refers to this record only. Voltage calibrations: 50 mV.

presynaptic terminals. For transferred TEA to be detected in the present experiments, by increase in spike duration, it must be present in much higher concentrations. It may be anticipated that the electrotonic junctions are distant from the perikarya, and that TEA entering the postsynaptic neurone will be transported by diffusion and/or retrograde axonal transport to the cell body, where it will be considerably diluted

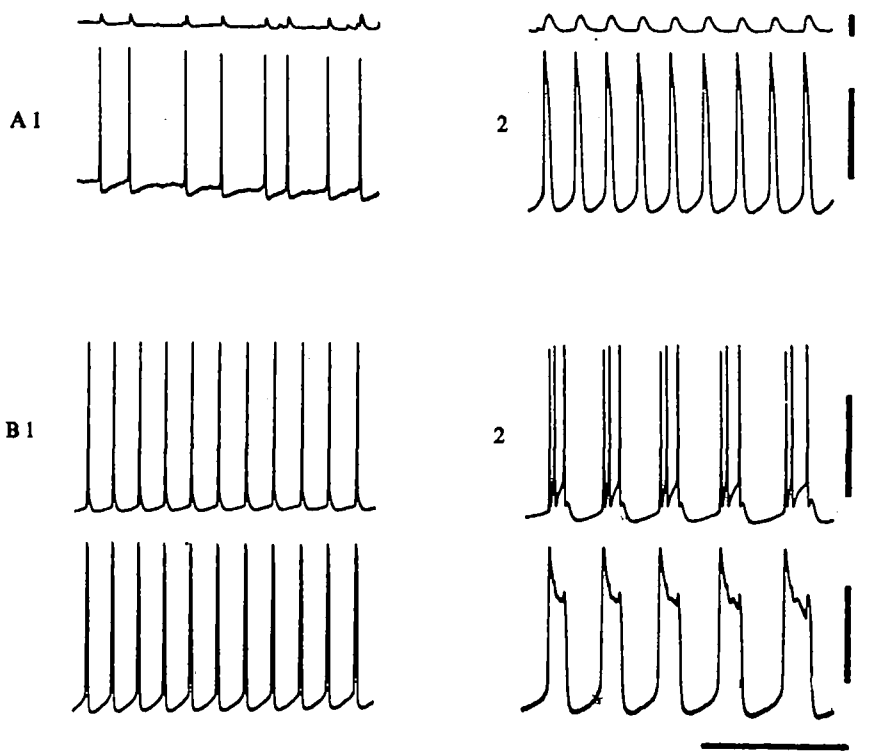

Fig. 3. Effect of presynaptic injection of TEA on synaptic transmission between the coupled neurones in the buccal ganglia. A and B are from different preparations. A 1, Action potentials elicited by maintained depolarization of the presynaptic neurone (lower) produce coupling potentials in the postsynaptic neurone (upper). A 2, After presynaptic injection of TEA for 1 hr, the coupling potentials are increased in amplitude and duration, though still subthreshold. B 1, After 30 min injection of TEA into the presynaptic neurone (lower) there is one-for-one transmission of action potentials to the postsynaptic neurone (upper). B 2, After 90 min injection of TEA, each presynaptic action potential results in the production of three postsynaptic action potentials. Time scale: 5 sec. Voltage calibrations: A (lower), B, 50 mV; A (upper), 2 mV.

because of the relatively large volume (cf. Deschênes & Bennett, 1974). The particularly long time which elapsed before effects were observed in the cerebral ganglion cells may be explained by the fact that they are in a group of coupled neurones, and it cannot be ascertained whether they are directly coupled.

The results show that TEA can cross electrotonic junctions in *Planorbis*. The postsynaptic changes are unlikely to be due to escape of TEA into the extracellular medium because of the large bath volume and continuous perfusion. In view of the large numbers of electrical synapses which have been described in molluscs (and many other invertebrates) the transfer of TEA across electrotonic junctions may present a drawback to the use of TEA for determining monosynaptic connexions. (Though it would appear that few electrotonic junctions show strict one-for-one transmission of action potentials when the presynaptic neurone is stimulated intracellularly. Spikes usually show considerable attenuation during transmission, due to the high frequency filtering characteristics of electrotonic junctions; they generally

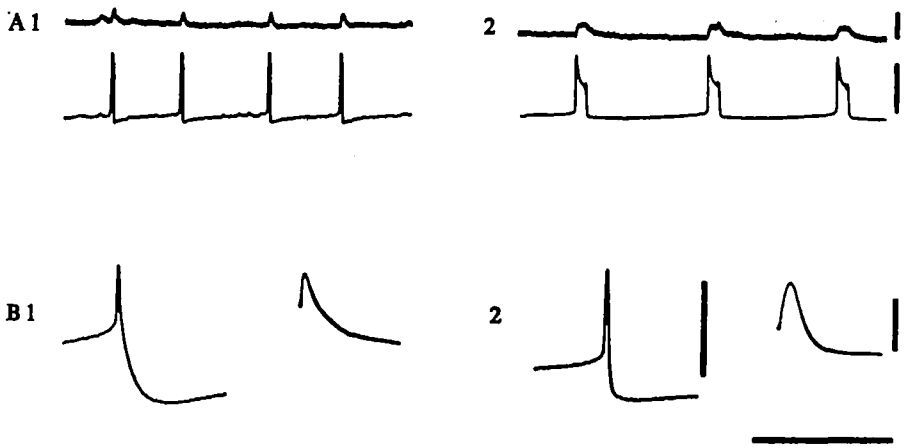

Fig. 4. Transfer of TEA between coupled neurones in the cerebral ganglia. The coupling is demonstrated in A. Action potentials elicited in the injected presynaptic neurone (lower) by maintained depolarization produce subthreshold coupling potentials in the postsynaptic neurone (upper). A1, TEA was injected for 30 min. A2, After injection for 90 min the coupling potentials are increased in amplitude and particularly in duration. B illustrates transfer of TEA between the same neurones. B1, Action potential in the postsynaptic neurone before presynaptic injection of TEA. B2, Action potential in the postsynaptic neurone 10 h after the start of a 3 h period of presynaptic injection of TEA. Spikes are recorded at two different speeds. Note the prolongation of the spike in B2, and the reduction of its undershoot, indicating transfer of TEA. Time scales: A, 5 sec; B1, B2 (first records), 240 msec; B1, B2 (second records), 50 msec. Voltage calibrations: A (upper), 2 mV; A (lower), B, 50 mV.

tend to be synchronized by virtue of the synchronization of slower potential changes such as pacemaker depolarizations and synaptic input.)

Another possible drawback to the use of TEA is illustrated in Fig. 3B. This is the possibility of repetitive firing in an intercalated neurone in response to single prolonged presynaptic spikes. This could occur at electrical or chemical synapses, particularly in neurones with low spike threshold. This possibility was noted by Kehoe (1972), who first used TEA as a test for monosynaptic connexions, but could be discounted because the latency of the postsynaptic potential was too short for such repetitive firing to cause the progressive increase in the postsynaptic response. It might be expected that repetitive firing in an intercalated neurone would be evident because more than one postsynaptic potential would be produced for each presynaptic spike. However, this would not be obvious where postsynaptic potentials show fairly smooth summation; this is not uncommon in molluscs, where postsynaptic responses often tend to be of long duration and to develop slowly. TEA is thus likely to give more clear cut results in cases where latency is short and discrete postsynaptic potentials are observed.

Although these reservations should be considered, TEA can provide important data on the likelihood of any particular synaptic connexion being direct or indirect; there are at present very few means available for making such a judgement.

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