Short communications

Effect of penicillin on the increase in membrane conductance induced by γ-aminobutyric acid at the crab neuromuscular junction

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The effects of penicillin and picrotoxin on the increase in membrane conductance produced by γ-aminobutyric acid (GABA) at the hermit crab neuromuscular junction were investigated. Penicillin failed to block the effects of GABA, while picrotoxin proved to be a potent antagonist.

It is well known that penicillin has an epileptogenic action when applied topically to the mammalian cerebral cortex (Ajmone-Marsan, 1969). Recently, Curtis, Game, Johnston, McCulloch & Maclachlan (1972) have suggested that this may be due to its ability to antagonize the inhibitory effects of γ-aminobutyric acid (GABA). There is considerable evidence that GABA is the inhibitory transmitter at the crustacean neuromuscular junction and this preparation has frequently been used as a model for GABA-mediated inhibition in the mammalian central nervous system (C.N.S.). This report describes the results obtained when penicillin was compared with picrotoxin, known to block GABA in crustacea (Earl & Large, 1972a ; Takeuchi & Takeuchi, 1969), at the crab inhibitory neuromuscular junction. Since GABA has been shown to mediate both pre- and postsynaptic inhibition at the crustacean neuromuscular junction (Dudel & Kuffler, 1961), in order to examine only postsynaptic events, we measured GABA-induced changes in muscle membrane conductance.

Methods.—The abductor muscle of large claws of hermit crabs (*Eupagurus bernhardus*) was used to study conductance changes produced by addition of GABA. The muscle was impaled with two separate intracellular microelectrodes, filled with 3M KCl ; one electrode was used for recording the voltage displacement produced by passing current pulses through the second electrode. Input resistance was read as the slope of the voltage-current plot, obtained when both electrodes were impaled in the middle of the fibre less than 100 μm apart. Using the corrected equations for a short cable (Weidmann, 1952), membrane conductance was calculated by the simplified method of Takeuchi & Takeuchi (1967). Readings were taken 1 min after the addition of each concentration of GABA. When studying the effects of penicillin and picrotoxin these compounds were left in contact with the preparation for 2 min before addition of the first dose of GABA. The Ringer composition was NaCl, 445 mM ; KCl, 12·2 mM ; $CaCl_2$, 29·6 mM ; $MgCl_2$, 5·75 mM and $NaHCO_3$, 1·79 mM. Substances used were γ-aminobutyric acid (GABA) (Sigma) picrotoxin (Sigma) and benzyl penicillin (Glaxo).

Results.—Increase in membrane conductance following addition of GABA to the bath was plotted against log concentration of GABA. An increase in input conductance became apparent with 1×10^{-5}M GABA and the maximal effect was obtained with $1\text{–}4\times10^{-4}$M.

In the presence of picrotoxin, GABA produced much smaller changes in membrane conductance (Fig. 1a), as previously described by Takeuchi & Takeuchi (1969). With increasing concentrations of picrotoxin, the maximal conductance change produced by GABA was progressively reduced, thus suggesting a non-competitive type of antagonism.

In 6 experiments, penicillin (1×10^{-4}–1×10^{-3}M) failed to antagonize the effect of GABA. The results of a typical experiment with 1×10^{-3}M penicillin are shown in Fig. 1b ; as can be seen, penicillin did not reduce the maximal conductance change. In contrast, while recording from the same fibre, subsequent addition of 1×10^{-5}M picrotoxin produced a marked effect.

Discussion.—These results show that GABA has a marked effect on the postsynaptic membrane of the hermit crab neuromuscular junction since it produces maximal conductance increases of the order of two to three-fold. Furthermore, this synapse resembles the inhibitory neuromuscular junction of the crayfish in that similar concentrations of picrotoxin

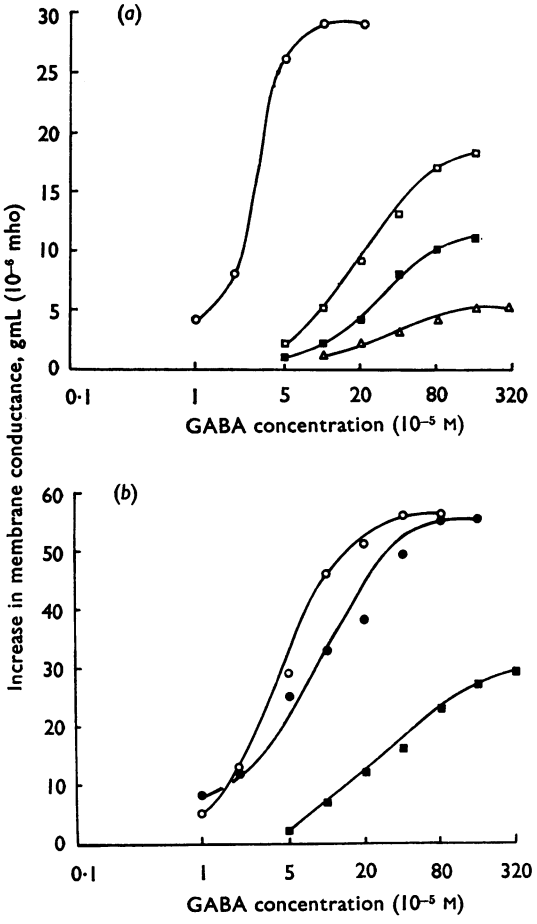

FIG. 1. Comparison of the effects of picrotoxin and penicillin on the relation between membrane conductance gmL (where gm is the membrane conductance of 1 cm of muscle fibre and L is the half-length of the fibre) and the concentration of γ-aminobutyric acid (GABA). (a) ○——○, Control; □——□, 5×10^{-6}M picrotoxin; ■——■, 1×10^{-5}M picrotoxin; △——△, 2×10^{-5}M picrotoxin. (b) ○——○, Control; ●——●, 1×10^{-3}M penicillin; ■——■, 1×10^{-5}M picrotoxin.

antagonize these increases in membrane conductance.

Our finding that penicillin does not block GABA at the crab neuromuscular junction does not necessarily contradict the findings of Curtis *et al.* (1972) that it was effective in cat C.N.S., because bicuculline, also a GABA antagonist in the mammalian C.N.S., has been shown to be relatively ineffective in crustacea (Earl & Large, 1972; Takeuchi & Onodera, 1972). Furthermore, we have shown that (+)-tubocurarine, reported to be a good GABA blocker in the cat C.N.S. (Hill, Simmonds & Straughan, 1972) is also without effect in our preparation (Earl & Large, 1973).

Therefore, it is becoming increasingly evident that the results obtained with possible GABA antagonists at the crustacean neuromuscular junction are often different from those observed in microiontophoretic studies in the mammalian C.N.S. One possible reason for this discrepancy may be suggested by considering the nature of antagonism. Our results with picrotoxin, and those of Takeuchi & Takeuchi (1969) strongly indicate a non-competitive type of antagonism of GABA. It is possible that the action of picrotoxin in blocking GABA in the mammalian C.N.S. (Obata, Takeda & Shinozaki, 1970) might also be mediated by a non-competitive mechanism. In this case the blocking

agent presumably combines with a site different from that of the agonist and therefore GABA-sensitive neurones in the mammal may possess sites for non-competitive antagonism which are not found in our preparation, although the GABA receptor itself could still be the same in both tissues. Alternatively, if bicuculline and penicillin antagonize GABA competitively in the mammalian C.N.S., our findings suggest that the GABA receptor is different from that at the crustacean neuromuscular junction. However it cannot be discounted that access of the antagonists to the receptors in the crab is in some way impeded.

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