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THE EFFECTS OF BRETYLIUM AND GUANETHIDINE ON CATECHOLAMINERGIC TRANSMISSION IN AN INVERTEBRATE

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1 Secretory potentials produced by stimulation of the salivary duct nerves were recorded intracellularly from cockroach isolated salivary glands. The secretory potential normally consisted of a 40-80 mV hyperpolarization of the gland cell.

2 Bretylium (0.1-1 mM) reduced the amplitude of the secretory potential without affecting the response of the gland to dopamine (0.25-1 μM). In addition, bretylium caused an increase in the frequency of miniature secretory potentials and the appearance of nerve terminal action potentials.

3 Guanethidine (1 mM) reduced the response to nerve stimulation without depressing the sensitivity of the salivary gland to dopamine (0.25-0.5 μM) and without causing an increase in the occurrence of miniature potentials. Higher concentrations (4-5 mM) completely eliminated secretory potentials but also reduced the sensitivity of the gland cell to dopamine.

5 These results indicate a presynaptic depression of the secretory potential by both bretylium and guanethidine. It is suggested that, in this system, bretylium acts by depolarizing the nerve terminal while guanethidine does not.

Introduction

Recent findings have suggested that a catecholamine, possibly dopamine, is released from the salivary nerve endings of the cockroach and acts to hyperpolarize the membrane of the salivary gland cell (Bland, House, Ginsborg & Laszlo, 1973; House, Ginsborg & Silinsky, 1973; Ginsborg, House & Silinsky, 1974). It would thus be of interest to investigate the effects of the adrenergic neurone blockers, bretylium and guanethidine, on the secretory potential (House, 1973) recorded from cockroach salivary glands in response to nerve stimulation. This paper describes such an investigation made on isolated preparations.

Methods

Salivary glands, ducts (with embedded nerves) and reservoirs were dissected from the cockroach *Nauphoeta cinerea* Olivier. The glands were spread across a transparent pedestal which emerged from the floor of a perspex bathing chamber (4 ml capacity). The preparation was secured to the pedestal by ligatures which were tied around reservoirs and connective tissue and then affixed to the chamber floor. Ducts were drawn into a suction electrode for stimulation of the encapsulated nerves. Trains of 50-500 stimuli (20-25 V) were delivered to the nerve at a frequency of 100 Hz every 2 minutes. Microelectrodes filled with 3M KCl and with resistances ranging from 10-30 MΩ were employed for intracellular recording from gland cells. The output of a preamplifier (W.P. Instruments) was fed in parallel into a Gould-Brush 220 pen recorder and a Tektronix Model 5103 N cathode-ray oscilloscope. The oscilloscope was used for monitoring nerve terminal action potentials visually. The records presented are photographs of pen recorder traces.

The normal bathing solution contained (mM) NaCl, 160; $CaCl_2$, 5; KCl, 1; $NaHCO_3$, 1; NaH_2PO_4, 1 (House *et al.*, 1973). Solutions were delivered to the preparation continuously at the rate of 2 ml/min by a Watson Marlow flow inducer and removed by suction. Solutions of drugs were applied by removing the delivery tube of the flow inducer from the control solution, placing it in a drug solution of the same ionic composition and briefly increasing the flow rate to 20 ml/min in

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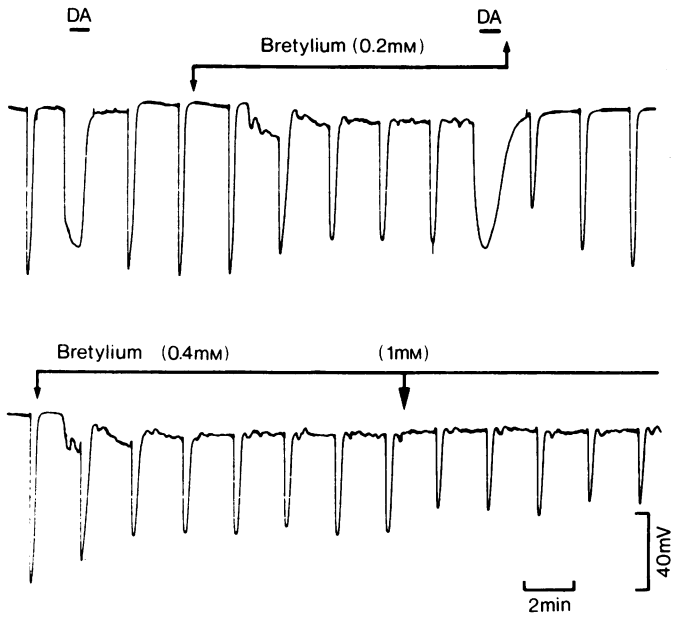

Fig. 1 Effects of bretylium on the response to nerve stimulation (secretory potential) and to dopamine, 0.25 μM (DA). The two records are continuous. Note that the presence of bretylium caused large increases in 'miniature' secretory potential activity. Resting potentials for cells in Figs 1-6 ranged from 35-50 mV (inside negative).

most experiments. Bretylium tosylate was obtained from the Wellcome Research Laboratories, guanethidine sulphate from CIBA and dopamine from the Sigma Chemical Company. All experiments were done at room temperature.

Results

Effects of bretylium

Figure 1 illustrates the effects of bretylium on the response to nerve stimulation and to dopamine, The secretory potential appears as a rapid, unlabelled downward deflection in all records. Figure 1 shows the secretory potential and the response to dopamine (0.25 μM) in the normal bathing solution. In the presence of bretylium (0.2 mM), the dopamine response remained unchanged while the secretory potential was reduced to 80% of control in this cell. When the concentration of bretylium was increased a further reduction occurred in the size of the secretory potential to 68% of control in bretylium (0.4 mM) and 51% of control in bretylium (1 mM) (Fig. 1), again without affecting the response to dopamine.

Similar results were obtained from other cells in different preparations. For example, bretylium (1 mM) in another preparation reduced the amplitude of the secretory potential to 41% of the control value. In four cells of different preparations bathed in bretylium (0.1 mM), responses to nerve stimulation were reduced to 70, 80, 89 and 93% of control. Concentrations much below 0.1 mM failed to produce an effect. The response to dopamine (1 μM) was unaffected by the presence of bretylium (1 mM) in two experiments.

In some cells, the depression of the secretory potential by bretylium (1 mM) could be overcome by increasing the numbers of stimuli while in other cells it could not, possibly because the test was made at a greater interval after the drug was applied (see below).

It is of interest that large increases in the frequency of 'miniature' secretory potentials (House, 1973) occurred in all six preparations bathed in bretylium solutions (see e.g. Figure 1). In one of these preparations in which eleven different cells were investigated, five cells bathed in the normal solution showed very infrequent spontaneous potentials while the six cells studied in the presence of bretylium revealed considerable miniature activity. Figure 2 presents a high gain record of miniature activity in one cell bathed in

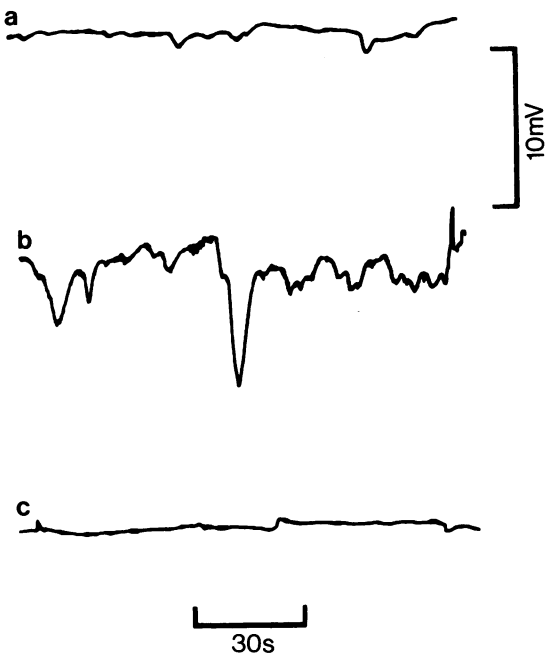

Fig. 2 Miniature secretory potential activity in the normal solution (a), in bretylium solution (b) and again in the normal solution (c). All records are from the same cell.

the normal solution (a), in bretylium (1 mM) (b) and again in the normal solution (c).

The results from three cells provided some insight into the possible cause of the increased miniature activity. In the experiment illustrated in Fig. 3, it may be presumed that the electrode was situated in close proximity to a nerve terminal. Figure 3a illustrates the miniature secretory potential activity in the normal bathing medium. After application of bretylium (1 mM) (Fig. 3), random nerve terminal action potentials (seen as spikes on the pen record) began to appear. These action potentials then dramatically increased in frequency until they seemed to generate a secretory potential of approximately 19 mV before the electrode was lost from the cell. Two other cells confirmed this result and also revealed that these action potentials were rapidly eliminated upon washing the preparation with the normal bathing solution. It should be noted that no stimuli were applied to the nerve in these three experiments nor was the flow rate altered when changing solutions.

Effects of guanethidine

Figure 4 illustrates the effects of guanethidine (1 mM). After approximately 21 min in guanethidine solution, when the secretory potential was reduced to 48% of control, a maximal response to dopamine (0.5 μM) was still observed. In two other preparations bathed in guanethidine (1 mM), secretory potentials were reduced to 52% and to 68% of control. In addition to depressing the response to nerve stimulation, higher concentrations of guanethidine produced a direct effect on the gland cell membrane as well. As Fig. 5 shows, guanethidine (4 mM) directly hyperpolarized the gland cell and reduced the amplitude of the secretory potential even though the preparation was rapidly washed with normal solution immediately after the hyperpolarization began. In two other cells, it was observed that guanethidine (4 mM and 5 mM) completely eliminated the secretory potential but this was accompanied by a reduction in the sensitivity of the gland cell to dopamine and by a direct hyperpolarization of the gland.

It is significant that none of five preparations

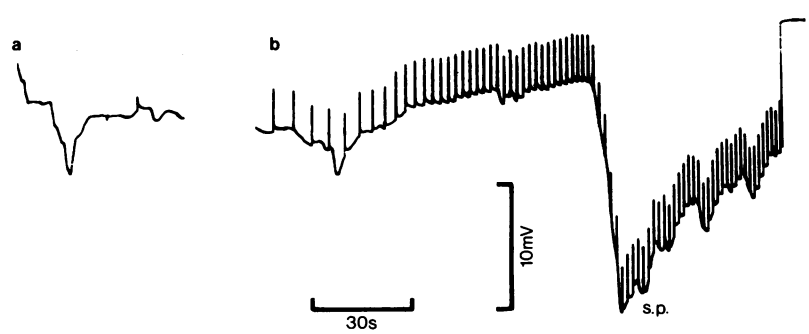

Fig. 3 Nerve terminal action potentials before (a) and during (b) treatment with bretylium 1 mM. s.p. secretory potential produced by bretylium in the absence of nerve stimulation. The action potentials appear as spikes in b.

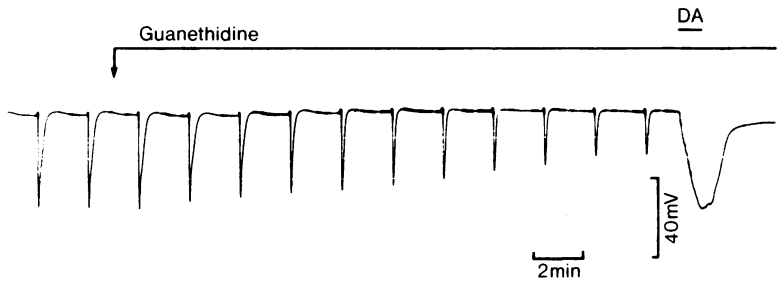

Fig. 4 Effect of guanethidine 1 mM on the response to nerve stimulation and to dopamine 0.5 μM (DA).

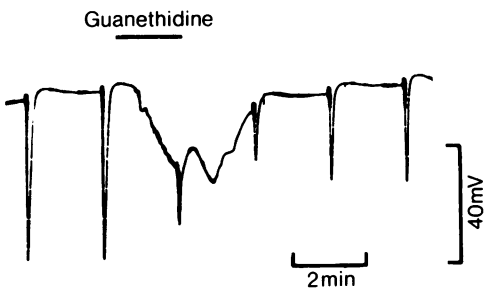

Fig. 5 Effects of guanethidine 4 mM. Note the large direct hyperpolarizing action as well as the reduction in the secretory potential.

Fig. 6 Surmountability of guanethidine-induced depression by increasing the number of stimuli. The first five secretory potentials were produced by 50 stimuli, the last (shown at increased recorder speed) by 500 stimuli. The guanethidine concentration was 1 mM.

bathed in concentrations of guanethidine ranging from 0.1 to 1 mM exhibited any increases in spontaneous activity. Concentrations of guanethidine below 0.1 mM were without affect.

During the early stages of blockade, the reduction in the size of the secretory potential could be overcome by increasing the numbers of stimuli. Figure 6 illustrates this effect, the recorder speed being increased in the last record to avoid confusion between the stimulus artifact and response peak. In the same cell, the depression of subsequent responses by guanethidine could not be surmounted by increasing the numbers of stimuli.

Discussion

In accordance with previous work on mammalian adrenergic systems (see review by Green, 1962), these results suggest that bretylium and guanethidine block catecholamine release from insect nerve terminals as well. The larger concentrations of these agents necessary to produce effects in the cockroach might be due to a relative insensitivity of dopaminergic systems to bretylium and guanethidine, or simply might indicate the fact that greater concentrations of drugs are necessary to reduce catecholamine release in insects.

The actions of bretylium described in this study are consistent with a depolarizing action of this drug on the insect nerve terminal. At the neuromuscular junction, depolarizing currents applied to the nerve endings produce large increases in miniature end-plate potential frequency (del Castillo & Katz, 1954; Liley, 1956) while reducing the amount of transmitter released by nerve impulses (Hubbard & Willis, 1968). Such an action would indeed explain the increased occurrence of miniature secretory potentials and the reduction in the response to nerve stimulation. Also consistent with this hypothesis is the appearance of nerve terminal action potentials in the presence of bretylium, the increase in the miniature secretory potential frequency in two cells appearing to be generated by a rapid increase in the frequency of these nerve impulses. That

these bretylium-induced action potentials originated in the nerve terminals and not in the axons is suggested by the lack of synchrony of the miniature secretory potentials. If bretylium acted directly on the nerve trunk, then a synchronous discharge of large secretory potentials would be expected rather than a scattering of smaller ones.

A significant difference between the depression caused by bretylium and that produced by guanethidine is that only the former was associated with increases in miniature activity and with the appearance of nerve terminal action potentials. These results suggest, therefore, that bretylium and guanethidine act differently from each other to produce the same ultimate effect, the presynaptic blockade of catecholaminergic transmission.

I would like to thank Dr B.L. Ginsborg and Dr C.R. House for their friendship and for introducing me to the rewarding aspects of cockroaches.

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