EFFECT OF PICROTOXIN ON ADRENAL CATECHOLAMINE SECRETION

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Abstract—The effect of picrotoxin (PT) on catecholamine (CA) secretion was investigated in perfused bovine adrenal glands. A low dose of PT (3 μM) enhanced the CA secretion evoked by a 15-min exposure to 1,1-dimethyl-4-phenylpiperazinium (DMPP, a nicotinic agonist; 0.1 mM), but a higher dose (0.3 mM) of PT inhibited the DMPP-evoked CA secretion. The rate of decline of secretory response to the prolonged DMPP stimulation was also accelerated by a higher dose (0.1 mM) of PT. In the dose-response curves for DMPP-evoked CA secretion, the inhibitory action of PT (0.3–1 mM) was more prominent at high doses than at low doses of DMPP. The inhibition pattern was similar to the pattern of a barbiturates blockade. In separate experiments, PT (0.1 mM) augmented calcium (10 mM)- and high potassium (56 mM)-evoked secretory responses. Spontaneous CA secretion was unaffected by PT at the concentrations indicated above. These results indicate that a low dose of PT potentiates, but higher doses inhibit, the adrenal CA secretion by a nicotinic agonist and that the inhibitory effect of PT resembles that of barbiturates.

Picrotoxin (PT) is well known as a GABA receptor antagonist (1) which blocks inhibitory neurotransmission mediated by GABA in the brain (2) and in the autonomic ganglia (3). The GABA receptor antagonism is the molecular basis for the potent convulsant action of the drug.

On the other hand, PT has been shown to inhibit nicotinic responses peripherally. In the experiments with perfused bovine adrenal glands, Sangiah et al. (4) found that PT inhibited acetylcholine-evoked catecholamine (CA) secretion more significantly than the CA secretion evoked by GABA. Such inhibition of the nicotinic response was also demonstrated in the autonomic ganglia (5). However, the mode of action of PT on the nicotinic response has been insufficiently investigated.

Interestingly, a depression of the nicotinic response has been shown in the adrenal medulla, using pentobarbital (6) which is another modulator of the GABA receptor system (7). Thus, it would be of interest to compare the mode of action of PT with that of a barbiturates blockade.

Based on these aspects, the present study was an attempt to reinvestigate the effect of PT on the adrenal CA secretion and characterize the inhibitory effect on the nicotinic response. As the result, we found that PT has a dual action on the adrenal CA secretion evoked by nicotinic agonists and that the inhibition pattern of PT is similar to that of barbiturates.

Materials and Methods

Fresh bovine adrenal glands were obtained at the local slaughter house and prepared for
perfusion as described previously (8). The glands were perfused retrogradely with Locke's solution (22–25°C, pH 7.2), using a multichannel metering pump (Harvard Apparatus Co., Inc.; Millis, MA). The flow rate was 4 ml/min. The experiments were started 40 min after the beginning of perfusion. The venous samples were collected at 3-min intervals, and CA was estimated fluorometrically (9). The composition of the Locke's solution was as follows (mM): NaCl, 154; KCl, 5.6; CaCl₂, 2.2; Na₂H₂PO₄, 2.15; NaH₂PO₄, 0.85 and glucose, 10. In calcium-free Locke's solution, CaCl₂ was omitted. High calcium or high potassium Locke's solution contained 10 mM CaCl₂ or 56 mM KCl with a decreased concentration of NaCl to maintain isotonicity. All these solutions were equilibrated with 5% CO₂ in O₂. The glands were stimulated by a continuous perfusion of Locke's solution containing stimulants during periods of 15 min, or by an infusion of stimulants into the perfusion fluid during a period of 3 min. Dose-response relationships were determined for DMPP (a nicotine receptor agonist) with three doses. The stimulants-evoked CA secretion was corrected for spontaneous secretion which was measured just prior to the stimulation. PT and barbiturates were present in the perfusion fluid 15 min before and during the stimulation. Spontaneous CA secretion (25.8±1.8 μg/3 min: n=43) was unchanged during perfusion of PT and barbiturates at the concentrations indicated.

The drugs were obtained from the following sources: amylobarbitone sodium (Nippon Shinyaku), barbitone sodium (Wako), 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP, Aldrich), mecamylamine hydrochloride (Sigma), pentobarbitone sodium (Tokyo Kasei), phenobarbitone sodium (Merck), picrotoxin (PT, Wako), thiopentone sodium (Tanabe).

Results

Dual action of picrotoxin: Figures 1 and 2 show the effect of PT on CA secretion evoked by a 15-min exposure to DMPP (0.1 mM). The CA secretion in response to the prolonged stimulation by DMPP was markedly enhanced in the presence of 3 μM PT. The total amount of CA for 15 min raised up to 1.9-fold of the control response (Fig. 1a). After the washout of PT, the response to DMPP was similar to the control response.

On the other hand, higher concentration (0.1 mM) of PT did not significantly alter the total amount of the DMPP-evoked CA secretion, but it accelerated the rate of decline of the secretory response to the prolonged DMPP stimulation (Fig. 1b). Following the washout of PT, the recovery response to DMPP was enhanced to the 1.8-fold of the control response as a total amount of CA. Furthermore, the secretory response was markedly inhibited in the presence of 0.3 mM PT. The total amount of CA was decreased to 5.8% of the control response. The inhibitory action by 0.3 mM PT was compatible to that of mecamylamine (0.1 mM), a nicotinic antagonist. Thus, PT at the dose of 3 μM augmented the secretory response to DMPP, but a higher dose (0.3 mM) inhibited it (Fig. 2).

Disposition of inhibitory actions of PT and barbiturates: Figure 3 shows the inhibitory action of PT on the dose response relationship for DMPP-evoked CA secretion. The glands were stimulated by a 3-min infusion of increasing concentrations of DMPP (0.04, 0.2 and 1 mM) at 3-min intervals. PT (0.3 and 1 mM) prevented the second or third CA secretion in response to higher doses of DMPP (0.2 and 1 mM), with less effect on the first secretory response.
Fig. 1. Effect of picrotoxin on DMPP-evoked catecholamine secretion from perfused bovine adrenal glands. Black bars show the DMPP stimulation and white bars the presence of PT. a), b): Typical examples.

to a low dose of DMPP (0.04 mM). Similar findings were obtained in the experiments with acetylcholine.

Figure 4 illustrates the inhibitory action of amobarbital on the dose response relationships for DMPP. Amobarbital (0.1 and 0.3 mM) markedly inhibited the responses to higher doses (100 and 300 µM) of DMPP (more than 65–95%). Similarly, pentobarbital, thiopental and phenobarbital (0.1 mM of
Fig. 2. Effect of picrotoxin on DMPP-evoked catecholamine secretion from perfused bovine adrenal glands. Each column shows total amount of catecholamine secretion evoked by a 15-min exposure to DMPP (0.1 mM) and the mean±S.E. of four experiments. Meca: mecamylamine. **: Significant difference from the control value (P<0.01).

Fig. 3. Picrotoxin blockade of DMPP-evoked catecholamine secretion from perfused bovine adrenal glands. Each value shows the mean±S.E. of four experiments. *, **: Significant difference from the control value (*P<0.05, **P<0.01).

Discussion
The adrenal CA secretion in response to DMPP was augmented by PT at a concentration as low as 3 µM. PT has been indicated to produce a rise in blood pressure and a pronounced hyperglycemia in subconvulsive doses (10). Thus, our finding may suggest
that PT enhancement of adrenal CA secretion might be physiologically relevant.

PT also augmented calcium- and high potassium-evoked CA secretion. Since a calcium-free condition and high potassium increase the membrane permeability to calcium (11-13), PT might promote an influx of calcium ions into the adrenal chromaffin cells. Although our study provides no direct information on the possibility, our results indicate that PT may facilitate some processes of excitation-secretion coupling in the chromaffin cells.

On the other hand, a higher dose (0.3 mM) of PT markedly inhibited DMPP-evoked CA secretion. Thus, PT has a dual action of potentiation (at low doses) followed by...
inhibition (at high doses) on the adrenal secretory response to a nicotinic agonist. The enhanced recovery response after the washout of the higher dose (0.1 mM) of PT might reflect that the potentiation by PT still remained in the tissue.

The inhibitory action of PT observed in this study was consistent with the report by Sangiah et al. (4). The present study further indicates the features of the inhibitory action in the dose-response curves for DMPP. The inhibitory effect of PT was more prominent at high doses than at low doses of DMPP. Namely, the inhibition pattern was insurmountable. The PT blockade was further compared with a barbiturates blockade since both drugs have been classified as modulators of the GABA receptor (1, 7). Pentobarbital has been shown to inhibit nicotinic secretory responses in the adrenal medulla (6). In our study, several barbiturates such as amobarbital, thiopental and phenobarbital also showed inhibitory effects on the nicotinic response. All of these agents markedly inhibited the CA secretion evoked by high doses but not low doses of DMPP, producing the insurmountable inhibition pattern in the dose-response curves for DMPP. A high dose of PT accelerated the rate of decline of the secretory response to prolonged DMPP stimulation. Barbiturates have been also shown to increase the rate of desensitization in the postjunctional membrane (14). Consequently, this feature of the inhibition of nicotinic response by PT was apparently similar to the case of a barbiturates blockade.

Recent biochemical studies (15) have demonstrated that both PT and barbiturates bind to the same receptor that is coupled to the complexes of the GABA receptor, benzodiazepine receptor and chloride ionophore. Sangiah et al. (4) suggested the presence of a GABA receptor in the adrenal medulla which mediates an increase in CA secretion. However, it remains unclear whether or not PT enhancement and blockade of adrenal CA secretion by nicotinic agonists are related to the action on the GABA receptor complexes in the adrenal medulla.

Conclusively, the present study indicates that a low dose of PT potentiates adrenal CA secretion in response to a nicotinic agonist, but high doses of PT inhibit the nicotinic response, resembling a barbiturates blockade.

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