Effect of piperazine on central and peripheral cholinergic synapses of the frog

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Summary. The anthelmintic agent piperazine did not antagonize carbachol-induced depolarizations of the frog sartorius muscle but depressed root potentials and acetylcholine release from the frog spinal cord. We suggest that piperazine has a presynaptic site of action at central cholinergic nerve-terminals.

Piperazine, an anthelmintic agent, paralyses *Ascaris* body muscles and can produce neurotoxic effects in humans when administered in large doses[3]. These side-effects consist of motor incoordination and muscle weakness. Although piperazine is a GABA agonist in invertebrates, it does not act so on vertebrate GABA receptors[4]. Previous studies, chiefly based on measurements of contractile responses, have shown that piperazine either potentiates[5] or blocks[5,6] peripheral cholinergic transmission. We examined the effects of piperazine on cholinergic synapses of 2 very simple in vitro preparations, the frog sartorius muscle and the isolated spinal cord. Extracellular recordings and, in the case of the spinal cord, determinations of endogenous acetylcholine (ACh) release were used.

Methods. Sartorius muscles were removed from frogs (*Rana temporaria*) and placed in a bath containing frog Ringer at room temperature. Extracellular recordings from the muscle surface were obtained with moving meniscus technique[7] via Ag/AgCl electrodes and displayed on a pen recorder. The isolated frog spinal cord (*R. temporaria*) was incubated in eserine-containing Ringer at 12 °C[8,9]. The 8th or 9th pair of spinal roots were used for stimulation and recording. The bathing medium was collected every 10 min and bioassayed for ACh on the leech dorsal muscle. At the doses used and for the short contact times (30 sec) piperazine did not interfere with the assay. Piperazine molarity is referred to the citrate salt.

Results. Bath-applied carbachol, a cholinesterase resistant cholinergic agonist, evoked concentration dependent depolarizations of the muscle (figure 1 inset) which reached a plateau within 3 min, allowing construction of the concentration-response curves (figure 1). In preliminary experiments a slow decline of sensitivity occurred such that repeated concentration-response curves were progressively flatter and had reduced maxima compared with the 1st curve. If this phenomenon is ignored, subsequent tests in the presence of piperazine may lead to the erroneous conclusion of non-competitive antagonism. In order to overcome this problem muscles were randomly divided into 4 groups in which the effects of carbachol alone, carbachol in the presence of piperazine (2 concentrations) or carbachol and tubocurarine were examined. Within each group a 6×6 latin square design[10] was adopted so that each carbachol concentration was tested in all positions of the 6 concentration sequence and that a different sequence was followed in each preparation. When these precautions were

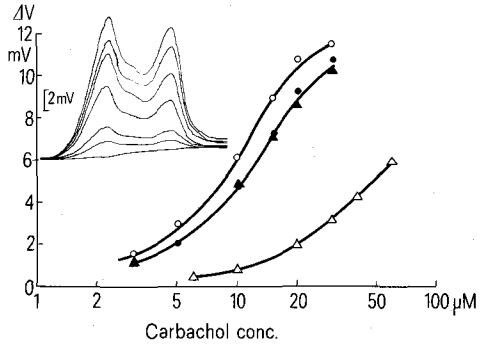

Fig. 1. Carbachol concentration-depolarization curves obtained from the in vitro sartorius muscle. Carbachol was bath applied and the surface electrical potentials measured 3 min later by moving meniscus technique[7,11]. 20 min elapsed between carbachol applications. When piperazine or tubocurarine were tested, they were equilibrated for 40 min before constructing carbachol concentration-response curve. A similar period was also allowed in control experiments. Abscissa: log-concentration of carbachol; ordinate: mV depolarization (ΔV). The points (different symbols) are the mean of 6 experiments (where SEM $\leq$ 15%); (●), control carbachol curve; (○), in the presence of 0.1 mM piperazine; (▲), in the presence of 0.5 mM piperazine; (△), in the presence of 1.44 μM tubocurarine. The inset shows original tracings obtained from the active areas of the muscle surface for increasing carbachol concentrations (3,5,10,15,20 or 30 μM). Note that these tracings closely correspond to those reported by Fatt[7] (cf. figure 2 of his paper) and that the left depolarization peak was used for the curves.

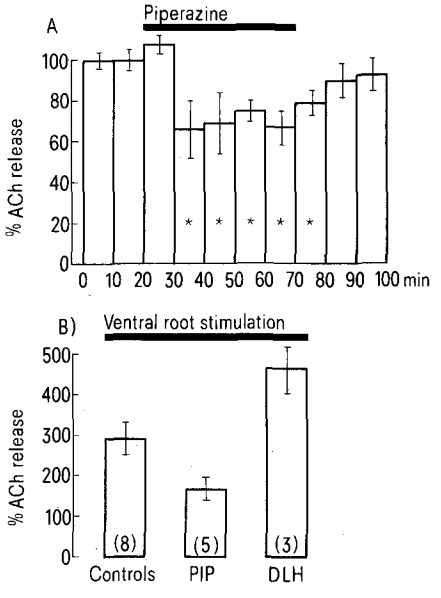

Fig. 2. Effect of piperazine on ACh release from the frog spinal cord. *A:* time course of depression of spontaneous ACh release following bath-application (filled bar) of 0.1 mM piperazine. The results are mean ± SEM (n = 15) and are expressed as percent variations of the control spontaneous release (100% = 16.7 ng · ml^{-1}. 10 min^{-1}) which is likely due to spontaneous tonic activity of the preparation[8,9]. The asterisks denote $p < 0.05$. *B:* effect of piperazine (PIP, 0.1 mM) or DL-homocysteate (DLH, 0.1 mM) on ACh release evoked by ventral root stimulation (1 Hz; 0.05 msec; supramaximal voltage for 10 min). The results (expressed as in A, with n in brackets) are calculated as percent of the sample just prior to stimulation. Both PIP and DLH results (obtained during the 3rd 10 min sample after drug application) were significantly different ($p < 0.05$) from stimulated controls. Note that unstimulated release in the presence of DLH was 175±21% of the control unstimulated value.

taken, piperazine (0.1 mM) slightly potentiated the carbachol responses whereas the higher concentration (0.5 mM) was ineffective (figure 1). Carbachol depolarizations were antagonized by tubocurarine which had a dissociation constant (average value = 0.41 μM, obtained by the dose ratio method) virtually identical to that calculated from the data of Jenkinson[11].

In the frog cord piperazine (0.1 mM) depolarized spinal roots (≤ 0.5 mV) and depressed (by 30–40%) the amplitude of the orthodromic ventral and antidromic dorsal root potentials. In unstimulated preparations piperazine produced a slowly-developing reduction (reversible on washing) of spontaneous ACh release, the peak effect being found 20 min after application of the drug (figure 2, A). 1 mM piperazine produced a similar depression with peak effect occurring in the 1st 10 min sample while a 50 μM concentration gave an insignificant decrease (10%) in ACh release. In order to check for a direct action of piperazine on functionally-identifiable cholinergic nerve terminals, ventral roots were stimulated antidromically for 10 min, commencing 30 min after application of the drug. Other cords were similarly stimulated, either in control Ringer or in the presence of the excitatory amino acid DL-homocysteate[13] (0.1 mM). Figure 2, B shows that piperazine reduced ACh release induced by antidromic stimulation whereas homocysteate produced an additive effect. This amino acid depolarized (about 0.5 mV) spinal roots and increased spontaneous ACh release by 75%.

Discussion. Piperazine had no antagonistic effect on nicotinic receptors of frog muscle although an inhibitory effect has been observed in other studies[5,6]. Since these authors measured a decrease in muscle contractions rather than changes in membrane potential, it is possible that piperazine antagonizes peripheral cholinergic transmission by acting not on postjunctional receptors but on contractile mechanisms. The slight potentiation of carbachol seen after 0.1 mM piperazine needs not to be interpreted as a change in cholinergic receptor sensitivity and is perhaps an unspecific action (e.g. due to an increase in membrane resistance). A presynaptic action of piperazine seems likely in view of its depressant effects on ACh release from the spinal cord. This effect is probably direct because it is present following antidromic ventral root stimulation which activates cholinergic[8,9,12] motor axon collaterals. It is not unlikely that the presynaptic action of piperazine contributes to the observed reduction in spinal cord potentials[4].

Axonal block cannot explain the reduced ACh release since the depression was less than that produced by the local anaesthetic tetrodotoxin[9] and large doses did not abolish evoked root potentials. Piperazine-induced reduction of electrically evoked release was larger than that calculated by simple subtraction of the spontaneous release in the presence of piperazine from the evoked release of untreated control cords. This implies that the release-depressing activity was greater during continuous electrical stimulation than at rest. Sustained nerve terminal depolarization reduces transmitter release due to nerve stimulation at the neuromuscular junction[14]. As piperazine and DL-homocysteate produced comparable depolarizations but had different actions on ACh release, it follows that ventral root depolarization did not reach the presynaptic terminals or was insufficient to depress release. In any case the action of piperazine on ACh release must have a different explanation. Since Ca^{2+} influx is a critical factor for ACh release[15], we might suppose that piperazine depressed release by interfering with presynaptic Ca^{2+} movements coupled with the excitation-secretion process.

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Interaction of chlorpromazine with organic solvents and fatty acids as studied by UV-spectrophotometry[1]

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Summary. The magnitude of the UV-spectral change of chlorpromazine increases in the presence of increasing concentrations of alcohols or fatty acids and with increasing chain length. A maximum is reached with 14.0- or 16.0-fatty acids. The difference spectrum is still larger with unsaturated fatty acids, a maximum effect being obtained with one cis-double bond. The spectral change is abolished by chaotropic and enhanced by antichaotropic agents.

Tissue-binding studies with basic lipophilic drugs[2] have revealed that the drugs used are mainly bound to microsomes and mitochondria of hepatic and extra-hepatic tissues. With chlorpromazine (CPZ) and related drugs, the binding could be further traced to membrane phospholipids, in particular to their fatty acid moieties[3]. Binding observed by equilibrium dialysis was confirmed by a spectroscopic technique, which is based on a red shift in the UV-spectrum of CPZ in the presence of both protein and lipid binders. Further investigations demonstrated that the magnitude of spectral change is dependent on the concentrations of both ligand and binder, that it is enhanced by a pH increase from 6 to 10, decreased by urea (8 M), and not changed by variation of the ionic strength from 0 to 0.5[4].