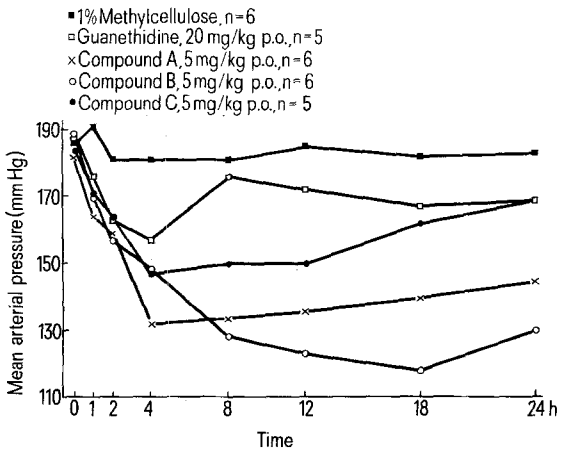

Fig. 2. Onset and duration of antihypertensive action of guanethidine and pyridinylidene arylureas in SH rats. N = number of animals per treatment group.

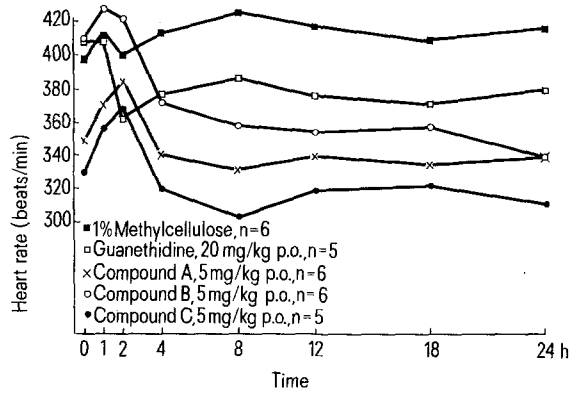

Fig. 3. Effects of guanethidine and pyridinylidene arylureas on heart rate in SH rats. Same experiments as in figure 2. N = number of animals per treatment group.

arylureas, papillary muscles from the right ventricle of cat hearts were isolated and electrically stimulated in accordance with the technique of Cattell and Gold[7]. None of the 3 compounds had significant myocardial depressant activity at 2.5 µg/ml although compounds A and B at 40 µg/ml reduced the isometrically recorded contractile force of papillary muscles (table 3). These findings suggest that the myocardial depressant effect is not likely to represent the major mechanism of the antihypertensive action of the pyridinylidene arylureas.

In preliminary behavioral studies in mice, all 3 compounds were tested at doses up to 150 mg/kg i.p. Compounds A and B but not C produced ataxia and ptosis and reduced exploratory behavior at doses below 50 mg/kg i.p. Compound C reduced exploratory behavior and produced ptosis only at doses exceeding 100 mg/kg i.p. Compound B was more toxic than compound A or C; its estimated 24-h LD_{50} in mice was 35.2 mg/kg i.p. whereas that of compound A or C was greater than 150 mg/kg i.p.

The findings in this study suggest that depletion of catecholamines represents a likely mechanism of the antihypertensive action of pyridinylidene arylureas. The relative inability of compound C to deplete brain catecholamines suggests that antihypertensive and central catecholamine depleting effects can be separated in this series of compounds and that compound C, while retaining antihypertensive activity, may be relatively free of CNS depressant properties of presently used catecholamine depleting agents, e.g. reserpine.

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Depression of spontaneous and ionophore-induced neurotransmitter release by *Salmonella*[1]

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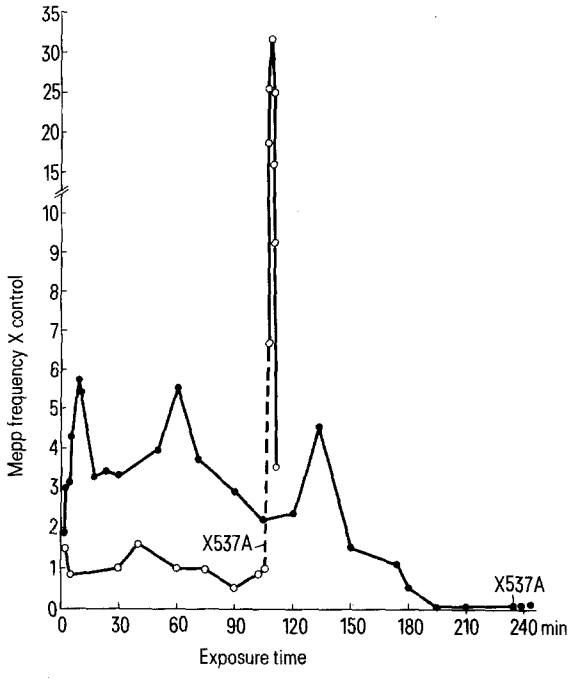
Summary. Exposure of frog neuromuscular junctions to heat-killed, lyophilized *Salmonella typhimurium* (SR 11) produces an early increase in spontaneous transmitter release followed by depression of release and blockade of the obligatory release usually induced by ionophore X537A.

Previous studies in our laboratory have shown that both crude and purified preparations of gram-negative bacteria depress neuromuscular transmission. Crude preparations of *Escherichia coli*, more commonly known as endotoxin, produce an increase, then a decrease in the frequency of miniature endplate potentials (MEPPs) recorded at the frog neuromuscular junction and block evoked endplate potentials (EPPs) by reducing quantal content[2]. The transient increase in MEPP frequency requires extracellular calcium. Purified preparations of the bacterial cell wall contain lipopolysaccharides (LPS) free of the protein contamination usually found in endotoxin. LPS similarly depresses spontaneous transmitter release but fails to produce the early, transient increase in MEPP frequency produced by endotoxin[3]. Sufficient exposure to LPS also apparently protects the presynaptic terminal from the usually destructive effects of the carboxylic cation ionophore X537A which, at untreated terminals, produces a transient rise in MEPP frequency to 100 times control levels followed by an abrupt abolition of both evoked and spontaneous transmitter release[4,5].

In general, the effect of LPS exposure seems to be one of



Effects of *Salmonella typhimurium* at concentrations of 10 (○——○ and 100 µg/ml (●——●) on the rate of spontaneous transmitter release at frog neuromuscular junctions. Release rate is shown as miniature endplate potential (MEPP) frequency times control frequency and time is measured from beginning of *Salmonella* exposure. Ionophore X537A (100 µM) was introduced at times indicated by arrows.

isolating the presynaptic terminal from extracellular space so that the terminal is no longer responsive to external agents including the calcium required for evoked transmitter release.

Methods. MEPPs were recorded from sartorius muscles of *R. pipiens* using conventional intracellular recording techniques with glass capillary microelectrodes as described previously[3]. Muscles were continuously perfused (2 ml/min through a 5-ml chamber) with normal Ringer's (NaCl, 115; KCl, 2.5; $CaCl_2$, 1.8; NaH_2PO_4, 0.69; Na_2HPO_4, 2.31; glucose, 5.5 mM/l) with the addition of 3 µg/ml neostigmine methylsulphate to inhibit cholinesterase activity. Ionophore X537A was added from a 100% ethanol stock solution to give a final concentration of 100 µM when required. Ethanol was maintained at constant levels in solutions control and experimental (0.4%) and had no effect on MEPP frequency. *S. typhimurium* (SR 11) was cultured locally, heat-killed, and lyophilized, and maintained dessicated. Stock solutions of 1 mg/ml in saline were mixed as required. Control MEPP frequencies were determined prior to the introduction of *Salmonella* over a 45-min period following a 1-h equilibration time for the muscle in the bathing solution.

Results. Junctions were monitored in a total of 5 preparations for an acceptable time after the introduction of *Salmonella*. In most experiments 2 junctions were monitored simultaneously for spontaneous activity. The figure 1 shows results from 2 experiments in which junctions were exposed to 10 and 100 µg *Salmonella*/ml bath. No change in MEPP frequency is evident after 100 min exposure to *Salmonella* at the lower concentration. Introduction of X537A (100 µM) after 106 min provoked the typical acceleration of MEPP frequency followed by abrupt decline; recording was terminated from this cell by muscle fasiculation evoked by ionophore-induced depolarization of muscle endplates. In contrast, in experiments in which junctions were exposed to *Salmonella* at a concentration of 100µ g/ml bath, MEPP frequency increased significantly (range 3–8 times control frequency) and, after a sufficient exposure time, spontaneous release rates declined to very low levels. In the experiment shown in figure 1, X537A was added after 235 min of exposure to *Salmonella* (100 µg/ml) and no effect of the ionophore was evident in increased MEPP frequency. Both muscle endplate depolarization and muscle fasiculation was reduced in most experiments.

Discussion. Exposure of neuromuscular junctions to heat-killed *S. typhimurium* leads to a mild but significant increase in the rate of spontaneous transmitter release. The increase is transient, though prolonged, and MEPP frequency eventually falls to very slow rates. In some experiments spontaneous release appeared to be entirely abolished.

This pattern is similar to that observed previously with endotoxin except for the considerable slowing of time course with the present unpurified material. We also observed that, after sufficient exposure at higher concentrations, *Salmonella* prevents the usually catastrophic response of the presynaptic terminal to the potent ionophore X537A. We previously found that the MEPP acceleration produced by endotoxin requires the presence of extracellular calcium which suggested that there may be a transient increase in calcium permeability across the membrane of the presynaptic terminal. A similar, induced alteration in calcium permeability should be suspected in the presence of sufficient concentrations of *Salmonella*. Increased calcium availability intracellularly and the resulting increased MEPP frequency is then followed by a decrease in transmitter release sufficient to reduce MEPP frequency to rates comparable to those usually observed in calcium-free media[2,6]. Since neither normal amounts of calcium nor high concentrations of X537A are capable of supporting or accelerating spontaneous transmitter release after prolonged exposure to *Salmonella*, we suspect that the presynaptic terminal becomes increasingly isolated from the extracellular environment.

The actual mechanism by which both heat-killed *Salmonella* and endotoxin produce transient acceleration of MEPP frequency remains undefined. We previously found that the degree of acceleration varied with different serotypes of *E. coli*. That portion of the crude preparation responsible for the acceleration is apparently lost in final purification to RNA-free LPS.

We suspect that, since the systemically toxic moiety of LPS is lipid A, the extremely lipophilic character of the moiety may result in partial or complete coating of the terminal membrane surface[7]. In the extreme, substitution of the bacterial LPS for a significant area of the terminal membrane may occur. Consequently, excitation-secretion coupling is abolished and the effect may be difficult to reverse. Such a functional isolation of the secreting membrane may well explain the refractory nature of advanced shock.

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