The Effect of Tetraethylpyrophosphate (TEPP) on the Transmembrane Potentials of Pacemaker and Non-pacemaker Fibers of Isolated Rabbit Atrium

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When $7 \times 10^{-7}$M tetraethylpyrophosphate (TEPP) was administered as constant perfusion, the results were: (1) A progressive slowing of atrial rate and subsequent block in conduction; (2) A decrease in slope of the prepotential of pacemaker fibers; (3) No significant change in membrane resting potential of sinoatrial and atrial fibers, even during block in conduction; (4) An accelerated repolarization of both types of fibers. Simultaneously with the changes in electrical activity TEPP consistently produced a progressive decrease in inotropism. Pyridine 2-aldoxime methiodide (PAM) in concentrations of 1.26-2.52 $\times 10^{-4}$M consistently reversed the effects of TEPP. The inotropism often exceeded the control. The data suggests that 1) the changes in transmembrane potential produced by TEPP are principally due to an acetylcholine action, 2) the decrease in contractility is more intimately related to a disturbance in metabolic energy, and 3) the reversibility of the TEPP effects by PAM is due to a reactivation of acetylcholinesterase, an action similar to that observed in vitro.

Hutter and Trautwein showed that stimulation of the vagi suppresses the rate of the pacemaker of the frog. While the heart was arrested the membrane resting potential (MRP) of the pacemaker fiber exceeded the control value. Vagal inhibition did not change the resting membrane potential of atrial fibers. On the other hand, vagal stimulation accelerated the repolarization phase of the action potential and reduced its amplitude and this effect was similar in sinus and atrial fibers. Hoffman and Suckling found no decrease in the magnitude of either the resting or action potential in atrial fibers of the dog following vagal stimulation.

Hutter and Trautwein reported that sympathetic stimulation increased the rate of rise of the upstroke and the amplitude of the overshoot were both increased. Repolarization was accelerated as a consequence of the increased rate.

Acetylcholine was shown by West, Falk, and Cervoni primarily to reduce the slope of the pacemaker prepotential and the magnitude of the action potential of the isolated rabbit atrium. In the atrial fiber of the dog Hoffman and Suckling observed that acetylcholine primarily enhanced repolarization, and not infrequently increased the rate of rise of the action potential. The magnitude of the action potential was often increased rather than reduced.

Van der Kloot reported that hexaethylpyrophosphate (HEPP), an inhibitor of cholinesterase, decreased the membrane resting potential in frog skeletal muscle irreversibly. Its effect on the membrane action potential was not obtained.

The purpose of the present study was two-fold; 1) to ascertain the effect of tetraethylpyrophosphate (TEPP), another anticholinesterase agent, on the membrane action potentials of pacemaker and non-pacemaker fibers...
TRANSMEMBRANE POTENTIALS OF PACEMAKER

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Action Potential</th>
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<tr>
<td></td>
<td>Maximal diastolic</td>
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<tr>
<td></td>
<td>(mV)</td>
</tr>
<tr>
<td>Pacemaker (N 18)</td>
<td>63±3</td>
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<tr>
<td>Nonpacemaker (N 18)</td>
<td>78±4</td>
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of the isolated right atrium of the rabbit; and 2) to evaluate the effectiveness of pyridine 2-aldoxime methiodide (PAM) in reversing the action of TEPP.

Methods

The anterior portion of the right atrium of the rabbit was incised, and the cut ends were pinned to a bar of wax in a lucite chamber. The endocardial surface was thus exposed. The tissue was bathed by a slowly moving Ringers-Locke solution into which 95 per cent oxygen and 5 per cent carbon dioxide were continually bubbled. The temperature of the bath was maintained between 34 and 36° C. The transmembrane potentials of pacemaker and nonpacemaker fibers were obtained by the ultramicroelectrode technic of intracellular recording. The sino-atrial pacemaker was usually located along a ridge of muscle which extended longitudinally between the orifices of the superior and the inferior vena cava. The atria were kept in the muscle chamber for at least one hour before initiation of an experiment. Tetraethylpyrophosphate (TEPP) was employed in concentrations of 3.5 X 10⁻⁷M to 7 X 10⁻⁷M and administered as a constant perfusion. Eighteen experiments were conducted in this series.

In the experiments employing pyridine 2-aldoxime methiodide the following modification in technic was employed. A portion of the superior margin of the right atrium was tied to a tension spring, and the opposite end was fastened to an adjustable holder. The holder was used to adjust the tension on the muscle. The changes in isometric tension were recorded from the spontaneously beating right atrium by means of a mechano-electronic transducer tube.6 Tension deflections on the recorder were calibrated with gram weights and the recording system was found to be linear in the range of tensions measured (0 to 5 Gm.). The transmembrane potential from the atrial fiber and the amplitude of contraction of the right atrium were recorded simultaneously. TEPP was administered until the heart beat ceased after which the flow of Ringers-Locke solution was re-instituted for 15 min. to ascertain if cardiac activity returned. If there was no recurrence of activity in this time then PAM (1.26-2.52 X 10⁻⁴M) was administered as a constant perfusion and the ability of this drug to reverse the effects of TEPP was observed. There were 12 experiments conducted in this series.

Results

Table 1 shows the mean and standard error values of the essential parameters of the transmembrane potentials of pacemaker and nonpacemaker fibers in 18 control experiments on rabbit atria. The temperature at which these experiments were conducted was between 34 and 36°C.

Effect of TEPP on Atrial Pacemaker and Nonpacemaker Fibers

When 7 X 10⁻⁷M TEPP was administered as a constant perfusion the usual results were: 1) a progressive slowing of atrial rate and
The effect of $7 \times 10^{-7}$M TEPP on the transmembrane action potential of the sinoatrial fibers of the isolated perfused right atrium of the rabbit.

Discussion in text. A, control; B, 13 min. after administration of $7 \times 10^{-7}$M TEPP as constant perfusion; C, 53 min. after; D, 60 min. after; E, 12 min. after return to control solution; F, 48 min. after return to control solution.

Time lines, zero level of potential and measurements of action potential as in Fig. 1.

subsequent failure of propagation; 2) a decrease in slope of the prepotential of the pacemaker fibers; 3) no significant change in the membrane resting potential of either fiber even during failure of conduction; and 4) accelerated repolarization and concomitant shortening of the duration of the action potential (APD) of both sinoatrial and atrial fibers. In 35 per cent of the experiments the magnitude of the action potential (MAP) decreased significantly with no appreciable change in the level of the membrane resting potential. The decrease usually occurred when the rate was appreciably slowed. Figures 1 and 2 are illustrative of the changes observed.

At lower concentrations ($3.5 \times 10^{-7}$M) the changes were similar but delayed. At the higher concentration ($7 \times 10^{-7}$M) the effects occurred often too rapidly to permit an adequate period of recording.

Effect of PAM on TEPP Treated Rabbit Atria

TEPP consistently produced a progressive decrease in contractility, simultaneous with the changes in electrical activity already described above (fig. 3).

PAM in concentrations of $1.26-2.52 \times 10^{-7}$M reversed the effects of TEPP. The return of the heart beat usually occurred within 10 min. Both the heart rate and amplitude of contraction increased with time and in the majority of instances both parameters returned toward control levels within 1 hour. Often the change in contractility exceeded the control (fig. 3). When PAM per se was administered in similar dosage (6 experiments) there was no consistent increase in the amplitude of atrial contraction.

Figure 4 is a graphic representation of the changes observed on both electrical and mechanical activity as modified by TEPP, and restored by PAM.
Discussion

The changes in the transmembrane potential of sinoatrial and atrial fibers of the rabbit atrium produced by TEPP are similar to those seen after vagal stimulation or the application of acetylcholine to pacemaker and nonpacemaker fibers of the frog or rabbit heart or the administration of diisopropyl fluorophosphate (DFP) to neural fibers of the frog. The similarity of the changes may be explained on the basis of an acetylcholine action. Both TEPP and DFP inhibit the acetylcholinesterase and this leads to an accumulation of acetylcholine. Similarly, vagal stimulation results in a liberation of acetylcholine at the motor end plate.

Harris and Hutter have shown that acetylcholine increases the permeability of the membrane to K. This may explain the enhanced repolarization caused by TEPP. Significantly, no decrease in membrane resting potential was observed in our study. This is at variance with the observations of Van der Kloofo noted above. He concluded that the decrease in the membrane resting potential was due to an inhibition of Na extrusion which correlated with the inhibition of the intracellular cholinesterase. The differences observed may be due to variations in technic, species difference, or differences in the chemical action of the 2 drugs.

The decrease in the magnitude of the action potential observed in 35 per cent of our experiments is more likely due to a decrease in membrane permeability to Na rather than an increase in permeability to K. However, it is not possible to explain the inconstant effect on the total magnitude of the action potential. In some experiments there was actually an increase in the magnitude of the action potential during slowing of the heart rate.

The block in conduction within the sinus venosus of the frog observed by Harris and Hutter following vagal stimulation has been explained as due to a decrease in the action potential to a point at which there is not enough local current for self propagation.

The normal level of diastolic membrane resting potential during conductive failure observed with TEPP may be ascribed to an inactivation of cellular esterase and alteration of protein receptor. The acetylcholine formed cannot act normally on the abnormal receptor resulting in an impaired transmigration of Na ions across the membrane and hence a failure to depolarize it. This is consistent with the hypothesis of Nachmannsohn on the function of the acetylcholine-cholinesterase system in neuro-muscular activity.

The changes in contractility produced by TEPP are similar to those reported by Roy and Kuperman. The dosage range employed in both studies was similar. The mechanism responsible for this effect may be attributed to an inhibition of cholinesterase, or to an interference with metabolic energy. Van der Kloot has shown that HEPP interferes with the supply of metabolic energy presumed necessary for the transport of Na across the membrane. The depressant effect of TEPP on contractility may therefore be due either to an interference with the utilization or with the supply of this energy, or with both.
The reversibility of the electrical and mechanical effects of the TEPP treated tissue observed with PAM is similar to that reported following the use of nicotinhydroxamic methiodide (NHMI) by Roy and Kuperman. Of the 2 drugs a smaller dosage of PAM was required to achieve the same result. These investigators also showed that the effect of NHMI was not the result of anticholinergic action since this compound did not reverse the effects of acetylcholine when administered to the rabbit atrium. That this reversal was not the result of a direct action of NHMI upon the tissue was shown by the insignificant response of the tissue to the compound per se. Our observations with PAM were similar. Both studies indicate that the reversibility of the effects due to TEPP on auricular tissue is probably due to a reactivation of cholinesterase similar to what is observed in vitro. Wilson demonstrated that the addition of TEPP to a solution of acetylcholine and acetylcholinesterase prevented the hydrolysis of acetylcholine. He postulated that the action of TEPP was due to its attachment on the active site of the enzyme. The addition of PAM to the solution containing TEPP inhibited enzyme resulted in a reactivation of the enzyme and hydrolysis of the acetylcholine. The mechanism of the reactivation of the enzyme by PAM was postulated by Wilson to be due to the removal of the TEPP bound to the active site of the enzyme by the formation of a phosphorylated oxime with PAM.

Summary

When 7 X 10^-3 M tetraethylpyrophosphate (TEPP) was administered as a constant perfusion, the usual results were: 1) A progressive slowing of atrial rate and subsequent failure of propagation; 2) a decrease in slope of the prepotential of pacemaker fibers; 3) no significant change in membrane resting potential of sinoatrial and atrial fibers, even during block in conduction; 4) an accelerated repolarization in both types of fibers. Simultaneously with the changes in electrical activity TEPP consistently produced a progressive decrease in contractility.

Pyridine-2-aldoxime methiodide (PAM) in concentrations of 1.26-2.52 X 10^-4 M consistently reversed the effects of TEPP. The restored contractility often exceeded the control in magnitude.

The data suggest that: 1) The changes in the transmembrane potential produced by TEPP are principally caused by acetylcholine; 2) the decrease in contractility is intimately related to a disturbance in metabolic energy and 3) the reversal of the effects of TEPP by PAM is due to a reactivation of acetylcholinesterase, as occurs in vitro.

Summario in Interlingua

Quando 7 X 10^-3 M de tetrnethylpyrophosphato (TEPP) esseva administrate a isolate preparatos de atri a conilio in le forma de un perfusion constante, le resultatos usual esseva: 1) Un progressive retardo del frequentia atrial e le subsequente discontinuation del propagation; (2) un reduction in le coefficiente directional del prepotential in le fibras del pacemaker; (3) nulle significative alteration in le potential de reposo del niembranas de fibras sinoatrial o atrial, mesmo in bloco de conduction; (4) un accelerate repolarisation in ambe typos de fibra.

In simultanetate con le alterationes del activitate electric, TEPP produceva uniformemente un progressiva redution del contractilitate. Pyridina-2-aldoxima-inethioduro (PAM) in concentrationes de 1,26 a 2,52 X 10^-4 M reverteva uniformemente le effectos de TEPP. Le restaurate contractilitate esseva frequentemente de un magnitude excedente le valor de controlo.

Le datos suggero quo: (1) le alterationes in le potential transinembranal que es producite per TEPP es causate principalmente per acetylcholine; (2) le redution del contractilitate es intimemente relacionate con disturbariones del energia metabolica, e (3) le reversion del effectos de TEPP effectuate per PAM resulta de un reactivation de acetylcholinesterase como illo ha esseva observate in vitro.

References

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