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INVESTIGATION OF THE ANTIFIBRILLATORY ACTIVITY OF SOME ANTICONVULSANT γ-AMINOBUTYRIC ACID-TRANSAMINASE INHIBITORS IN THE RABBIT ISOLATED HEART: COMPARISON WITH PHENYTOIN AND MEXILETINE

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1 The electrical stimulation and ventricular fibrillation thresholds were measured in the Langendorff-perfused rabbit heart by application of rectangular impulses of 3 ms duration and increasing current at frequencies of 4 and 20 Hz respectively.

2 Perfusion with either phenytoin or mexiletine produced significant dose-dependent increases in both thresholds.

3 Perfusion with four anticonvulsant inhibitors of γ-aminobutyric acid (GABA)-transaminase, amino-oxyacetic acid, γ-acetylenic GABA, γ-vinyl GABA and sodium valproate, up to a concentration of 20 μg/ml, had no significant effect on either threshold.

4 The results suggest that these compounds, unlike phenytoin and mexiletine, are devoid of antifibrillatory activity.

Introduction

The possible similarity between cerebral impulses in epilepsy and ectopic activity in myocardial infarction was noted by Harris & Kokernot (1950) who were able to demonstrate an antiarrhythmic effect of the antiepileptic agent, phenytoin (diphenylhydantoin). Subsequently, phenytoin has been proved to be effective in correcting various experimental and clinical arrhythmias (see review by Dreifus & Watanabe, 1970) and has gained wide clinical use. Mexiletine (Kö 1173) is another example of a drug originally introduced as an anticonvulsant agent but in subsequent studies found to be effective in suppressing ventricular arrhythmias both in experimental animals (Allen, Kofi Ekue, Shanks & Zaidi, 1972) and in man (Talbot, Clark, Nimmo, Neilson, Julian & Prescott, 1973). In recent years, compounds such as amino-oxyacetic acid (Murakami, Abe & Murakami, 1976) and sodium valproate (Simler, Ciesielski, Maitre, Randrianarisoa & Mandel, 1973) have been found to possess anticonvulsant action, an effect which was partly attributed to their action of elevating brain levels of the inhibitory transmitter, γ-aminobutyric acid (GABA). This elevation of GABA levels was found to be due to their inhibition of GABA-transaminase (GABA-T), an enzyme involved in GABA degradation. This latter finding has led to the synthesis of two catalytic inhibitors of GABA-T, γ-acetylenic GABA (Jung, Lippert, Metcalf, Schechter, Bohlen & Sjoerdsma, 1977) and γ-vinyl GABA (Jung, Lippert, Metcalf, Bohlen & Schechter, 1977). These two new compounds have also proved effective in protecting against seizures induced by different methods in mice (Schechter, Tranier, Jung & Bohlen, 1977; Schechter, Tranier, Jung & Sjoerdsma, 1977) and baboons (Horton & Meldrum, 1978).

Accordingly, it was thought worthwhile to investigate the effect of these four relatively new anticonvulsants on the electrical stimulation and ventricular fibrillation thresholds of the Langendorff-perfused rabbit heart and to compare their effect with that of phenytoin and mexiletine, two drugs possessing both anticonvulsant and antifibrillatory activity.

Methods

Perfusion and recording

New Zealand White rabbit of either sex weighing 1 to 1.7 kg were killed by a blow on the head and bled. The heart was rapidly removed and placed in McEwen's solution (1956), gassed with 95% O_2 and 5% CO_2 at room temperature. The aorta was immediately cannulated and coronary perfusion was started at a pressure of about 6 KPa (60 cmH_2O) at 37 C. Perfusion was with pure McEwen's solution of the

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following composition (mmol/l): NaCl 130, KCl 5.6, $CaCl_2$ 2.2, NaH_2PO_4 0.9, $NaHCO_3$ 25, glucose 11 and sucrose 13 or this solution with a drug could be selected by turning a tap. The amplitude of contraction was recorded on one channel of an oscillograph via a small stainless steel hook passed through the ventricular apex and connected to a force displacement transducer. Two silver–silver chloride wires were connected to the heart through two wick electrodes which were placed on the anterior surface of the base of the two ventricles to record the electromyogram. This was displayed on an oscilloscope (Airmec type 279) and was recorded on a second channel of the oscillograph. The heart was stimulated through the hook attached to the apex and another connected to the base of the left ventricle. In order to test whether there was likely to be mechanical trauma affecting the threshold of the tissue in which the former hook was fixed, some experiments were carried out in which a third hook, near the apex and not attached to any transducer, was used as a stimulating electrode alternately with the apical hook. The results showed that the thresholds did not vary according to which of these two hooks was used as an electrode and so it was decided to use the one attached to the apex throughout the study and save piercing the heart by an extra hook. The stimulating electrodes were linked to an isolated battery-powered constant current stimulator. The current multiplier control was turned by a d.c. motor and gearbox to increase the current intensity at a constant rate.

Measurements

After a period of at least 40 min of spontaneous beating, allowed for coronary flow, heart rate, amplitude of contraction and electromyogram to stabilize, two measurements were made:

Table 1 Effects of phenytoin, mexiletine, amino-oxyacetic acid, γ-acetylenic GABA, γ-vinyl GABA and sodium valproate on electrical stimulation threshold (EST) of the isolated heart of the rabbit

Concentration (*μmol/l*)	*% change in EST after exposure for* 15 min	*60 min*	*% change in EST after washout for* 15 min	*60 min*
Control	−5 ± 2.9	−3.3 ± 2.7	−1.5 ± 2.5	−3.4 ± 2.3
Phenytoin				
3.65	−0.8 ± 5.8	+14.8 ± 5.1*	+9 ± 5.9	+10.2 ± 6.1*
9.1	+8.5 ± 3.5*	+22.3 ± 5.4**	+9.8 ± 2.9**	+8.2 ± 3.4*
18.2	+19 ± 6.8*	+84 ± 10**	+59 ± 14**	+61 ± 16**
Mexiletine				
4.64	+15.3 ± 2.3**	+25.2 ± 8.4**	+1.7 ± 3.8	+4.5 ± 5.9
8.1	+41 ± 15	+62 ± 11.9*	+19 ± 9.8	+11.8 ± 13
11.6	+76 ± 14*	+128 ± 27*	+62 ± 24*	+43 ± 21
Amino-oxyacetic acid				
45.7	−0.8 ± 1.8	−0.4 ± 3.1	+0.8 ± 2.7	−3.2 ± 1.3
91.5	−3.2 ± 5.1	−0.8 ± 8.4	+6.2 ± 8.1	+6.2 ± 8.1
183	+2 ± 2.2	+3.8 ± 7.7	+1 ± 2.4	+1 ± 2.4
γ-Acetylenic GABA				
39.3	−3.6 ± 3.3	−4.3 ± 4.5	+0.4 ± 3.4	−2.7 ± 3.4
78.6	+0.4 ± 2.6	+1.2 ± 6.1	−2.4 ± 5.9	−0.4 ± 7.8
157	−5 ± 5.3	−2.3 ± 5.7	+1 ± 6.3	+1 ± 6.3
γ-Vinyl GABA				
38.7	−0.6 ± 1.8	−0.4 ± 1.9	−1.8 ± 2.8	−1.8 ± 2.8
77.4	+2.7 ± 1.9	+0.7 ± 1.6	−1.3 ± 1.2	−1.7 ± 1.1
155	−3.3 ± 3.2	−6.7 ± 4.2	−6.1 ± 3.8	−6.1 ± 3.8
Sodium valproate				
30.1	−6.4 ± 2.7	−4 ± 4.1	−4.2 ± 4.9	−4.2 ± 4.9
60.2	+2 ± 1.2	+0.8 ± 1.8	+3.6 ± 3.5	−2 ± 3.4
120	+1.8 ± 10.7	−1.6 ± 7.9	−3.8 ± 7.1	−4.8 ± 7.4
481	−2.6 ± 4.6	−2 ± 6.5	+4 ± 8.3	+3.6 ± 6.3

Values are means ± s.e. of 8 experiments for control and 5 or 6 for each concentration of each drug.
*$P < 0.05$; **$P < 0.01$.

(1) *Electrical stimulation threshold (EST)* Rectangular impulses of 3 ms duration at a frequency of 4 Hz were applied to the heart. The current was increased at a rate of 30 μA per second until the heart followed the stimulus. The minimum current required to drive the heart was taken as the EST. After this threshold was determined, stimulation was stopped and the heart resumed spontaneous beating.

(2) *Ventricular fibrillation threshold (VFT)* Serial rectangular impulses of 3 ms duration at a frequency of 20 Hz were used. The current was increased at a rate of 30 μA per second. The minimum current required to induce ventricular fibrillation was taken as the VFT. The moment fibrillation was induced, stimulation was stopped and if normal rhythm had not returned in 60 s, defibrillation was effected by infusing 0.1 ml of 0.54 mol/l potassium chloride into the aortic cannula. In each experiment three determinations of each threshold were carried out at 15 min intervals and the mean was taken as the control value. The heart was then perfused with McEwen's solution containing the test drug and both thresholds were determined after 15, 30 and 60 min exposure to the drug. The heart was then reperfused with the drug-free solution and similar determinations were carried out after 15, 30 and 60 min.

Drugs used were phenytoin sodium (Parke-Davis), mexiletine hydrochloride (Boehringer), amino-oxyacetic acid (Sigma), sodium valproate (Reckitt & Colman), γ-acetylenic GABA and γ-vinyl GABA (Recherche Merrell International). The statistical significance of differences was calculated by Student's paired *t* test.

Table 2 Effects of phenytoin, mexiletine, amino-oxyacetic acid, γ-acetylenic GABA, γ-vinyl GABA and sodium valproate on ventricular fibrilation threshold (VFT) of the isolated heart of the rabbit

Concentration (μmol/l)	*% change in VFT after exposure for*		*% change in VFT after washout for*	
	15 min	*60 min*	*15 min*	*60 min*
Control	−14.4 ± 4.1	−10.5 ± 3.8	−8.6 ± 6.3	−13 ± 6.6
Phenytoin				
3.65	+40.6 ± 3.1*	+28 ± 5.6*	−20 ± 7.1	−25.2 ± 8.3
9.1	+128 ± 11*	+141 ± 19*	−23.8 ± 6.4	−24 ± 8.3
18.2	+299 ± 42**	+318 ± 33**	−8.3 ± 12.6	−7.8 ± 13.6
Mexiletine				
4.64	+66 ± 9.9**	+51 ± 4.2**	−15.2 ± 7.3	−21 ± 11
8.1	+134 ± 17*	+118 ± 19*	−17 ± 9.7	−14 ± 15.3
11.6	+256 ± 35*	+263 ± 29*	+6.4 ± 10.6	−2 ± 10.7
Amino-oxyacetic acid				
45.7	+13.2 ± 20	+4.6 ± 18	+5 ± 12.4	+5.4 ± 10.4
91.5	−2.8 ± 2.9	−2.8 ± 7.6	−5.8 ± 6.6	−3.8 ± 7.3
183	+3.1 ± 9.2	+25.4 ± 17.3	−11.3 ± 7.8	−4.8 ± 3.8
γ-Acetylenic GABA				
39.3	−5.1 ± 5.3	−9.3 ± 6.3	−14.4 ± 16.5	−12.6 ± 17.4
78.6	−25.4 ± 8.6	−24.8 ± 8.2	−15.4 ± 8.4	−15.8 ± 7.4
157	−37.8 ± 7.9	−30.1 ± 11.5	−6.6 ± 13.5	−2.6 ± 13.3
γ-Vinyl GABA				
38.7	−3.8 ± 3.5	−15.1 ± 4.2	−11.6 ± 3.6	−13.8 ± 3.9
77.4	−13.2 ± 11.8	−12.2 ± 12.8	−15.4 ± 17.3	−17.2 ± 4.7
155	−17.3 ± 3.3	−21.6 ± 4.2	−18.4 ± 5.9	−18.2 ± 9.7
Sodium valproate				
30.1	−2.4 ± 2.6	−8.2 ± 4.2	−14.6 ± 5.2	−17.4 ± 5.5
60.2	−2.2 ± 1.9	−4.2 ± 5.2	−6.8 ± 4.7	−7.4 ± 4.8
120	−3.4 ± 9.3	−17.8 ± 8.6	−24 ± 14.4	−17.8 ± 13.9
481	−9.3 ± 6.2	−12.4 ± 11	−15.4 ± 9.5	−12.5 ± 8.7

Values are means ± s.e. of 8 experiments for control and 5 or 6 for each concentration of each drug.
*$P < 0.01$; **$P < 0.001$.

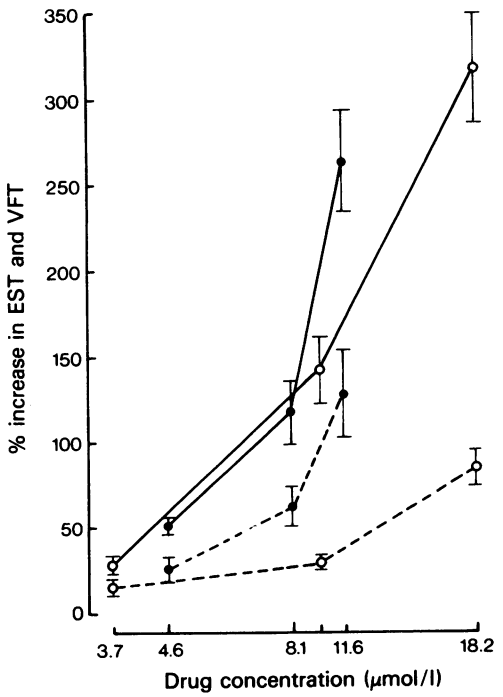

Figure 1 Effects of phenytoin (○) and mexiletine (●) on electrical stimulation threshold (EST, dotted lines) and ventricular fibrilation threshold (VFT, complete lines) measured in the rabbit isolated heart. Abscissa scale: drug concentration on logarithmic scale. Ordinate scale: percentage increase in EST and VFT. Each point is the mean of six experiments; vertical lines show s.e. mean.

Results

In 97 rabbit hearts used in the study, EST values ranged between 0.1 and 0.55 mA and VFT values between 0.3 and 9.5 mA. To test the stability and reproducibility of the method, both thresholds were determined on eight hearts in the absence of any drug throughout the experiments. In all these, within 1 h of the first determination, EST dropped by 1.5 to 5% and VFT by 8.6 to 14.4% of their original values and remained within these limits for another 2 h. The percentage changes in EST and VFT in the untreated hearts and those produced by each drug are summarized in Tables 1 and 2 respectively. During perfusion with either phenytoin or mexiletine there were significant, dose-dependent increases in EST and VFT. Dose-response curves of the effect of phenytoin and mexiletine, after an exposure duration of 60 min, on both thresholds, are plotted in Figure 1. These curves show that the elevation by both drugs of VFT is 2 to 4 times the rise in the corresponding EST. This effect of both drugs on VFT was completely removed within 15 min of reperfusion with the drug-free solution, while the effect on EST was not.

On the other hand, perfusion with the four newer anticonvulsants, amino-oxyacetic acid, γ-acetylenic GABA, γ-vinyl GABA and sodium valproate, up to a concentration of 20 μg/ml, did not produce any significant change in either EST or VFT. An additional concentration of 80 μg/ml of sodium valproate was also tested since the peak serum level in treated patients has been reported to be 71.8 ± 9.3 μg/ml (Perucca, Gatti, Frigo, Crema, Calzetti & Visintini, 1978). This concentration had no effect on either threshold. With the other three compounds no higher concentrations were tested since, up to date, there have been no studies in which their antiepileptic effects were correlated with plasma or CSF concentrations.

Discussion

Since Wiggers & Wegria (1940) introduced the concept of electrical vulnerability to fibrillation, determination of the fibrillation threshold, with a variety of modifications of the original method, has been one of the widely used techniques in the experimental assessment of antifibrillatory activity. In the present study, serial impulses of constant duration and increasing current were applied to the isolated perfused heart of the rabbit until ventricular fibrillation occurred. In addition, the electrical stimulation threshold, which is an indication of the heart excitability, was also measured. In the absence of any drug during 3 h of perfusion, neither threshold altered significantly. Perfusion with phenytoin or mexiletine produced significant, dose-dependent increases in EST and VFT. The greater effect of both drugs in elevating VFT in comparison with their effect on EST suggests that VFT determination is a more sensitive and selective test of antifibrillatory activity. However, the reason why this large effect on VFT was entirely removed upon reperfusion with the drug-free solution while that on EST was not, is not clear, bearing in mind that in control experiments neither threshold increased above the control value. The elevation of both thresholds by phenytoin was expected since Singh & Vaughan Williams (1971) found, in an electrophysiological study, that phenytoin reduced the maximum rate of depolarization. Therefore they concluded that its mode of action is not fundamentally different from other 'class 1' antiarrhythmic drugs such as quinidine, procainamide and lignocaine (Vaughan Williams, 1970). The same authors (Singh & Vaughan Williams, 1972) have found mexiletine to possess similar electrophysiological properties.

In contrast, none of the newer anticonvulsants, amino-oxyacetic acid, γ-acetylenic GABA, γ-vinyl GABA and sodium valproate was effective in raising EST or VFT. The highest concentration of amino-oxyacetic acid seemed to cause a slight increase in VFT, whereas that of γ-acetylenic GABA and γ-vinyl GABA appeared actually to decrease VFT to a level lower than that of the control group, but neither effect was statistically significant. The results indicate that these compounds, unlike phenytoin and mexiletine, are devoid of antifibrillatory activity. This is in accord with the finding that their anticonvulsant action is not due to a membrane stabilizing effect like that of phenytoin, but due to other effects such as elevation of brain levels of GABA (Simler *et al.*, 1973; Murakami *et al.*, 1976; Schechter *et al.*, 1977).

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