POTENTIATION OF THE ANTIARRHYTHMIC ACTION OF LIDOCAINE BY TETRODOTOXIN IN THE LATE STAGE OF EXPERIMENTAL MYOCARDIAL INFARCTION

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The mechanism of the antiarrhythmic action of lidocaine, a preparation introduced into clinical practice more than 30 years ago, has not yet been explained. Many investigations have shown that lidocaine in therapeutic concentrations, not changing the velocity of conduction of excitation along fibers of the conducting system and contractile myocardium [7, 8], has the property of inhibiting pacemaker activity and of shortening the duration of the action potential and the refractory period of Purkinje fibers [7, 8, 14]. The antiarrhythmic action of lidocaine is connected with these electrophysiological effects. More recently, however, observations have been made which show that lidocaine in therapeutic concentrations selectively reduces the velocity of spread of excitation in the zone of ischemia [9, 10]. Furthermore it has been shown that lidocaine, like tetrodotoxin (TTX), a specific blocker of fast sodium channels, blocks the conduction of excitation in fragments of myocardium isolated from the zone of an infarct [12]. These findings suggest that the antiarrhythmic action of lidocaine in myocardial infarction is connected with a decrease in the fast inward sodium current in the zone of ischemia.

To test this hypothesis, in the investigation described below the possibility of potentiating the antiarrhythmic action of lidocaine in the late stage of experimental myocardial infarction by combined administration of the drug with TTX was studied.

EXPERIMENTAL METHOD

Experiments were carried out on mongrel dogs of both sexes weighing 10-15 kg. Under pentobarbital anesthesia (35 mg/kg, intravenously) a myocardial infarct was induced by two-stage occlusion of the left descending coronary artery by Harris' method [1]. The ECG (lead II) and the bipolar electrogram from the auricle of the right atrium were recorded 24 h after the operation under chloralose anesthesia (100 mg/kg, intravenously), under artificial ventilation conditions. Recordings were obtained for periods of 1 min, at intervals of 10 min, for 1 h and the mean frequency of ventricular extrasystoles per minute and their percentage of the general cardiac rhythm were determined. If marked and lasting ventricular extrasystoles were present, and if the fluctuations in the frequency of the extrasystoles did not exceed 10%, intravenous injections of TTX and lidocaine were given. The ECG and electrogram were recorded before and during the injection and throughout the period of action of the drugs.

The initial concentrations were 1 μg/kg of TTX and 4 mg/kg of lidocaine. In the absence of an antiarrhythmic action, the concentrations of the drugs were increased in steps of 1 μg/kg of TTX and 4 mg/kg of lidocaine, until minimal doses of TTX and lidocaine causing a marked antiarrhythmic effect, described as threshold doses, were ascertained (the frequency of the extrasystoles was reduced by more than 50%). Doses of TTX and lidocaine of half the threshold values had practically no effect on the arrhythmias. These doses were taken to be subliminal. After the threshold and subliminal doses of TTX and lidocaine had been established, the antiarrhythmic action of a mixture of subliminal doses of these drugs was tested. In all experiments the drugs were dissolved in 5 ml of physiological saline and injected in the course of 1 min. Intervals between injections amounted to 1 h (10-40 min after injection of the threshold doses the original level of the arrhythmias was restored).

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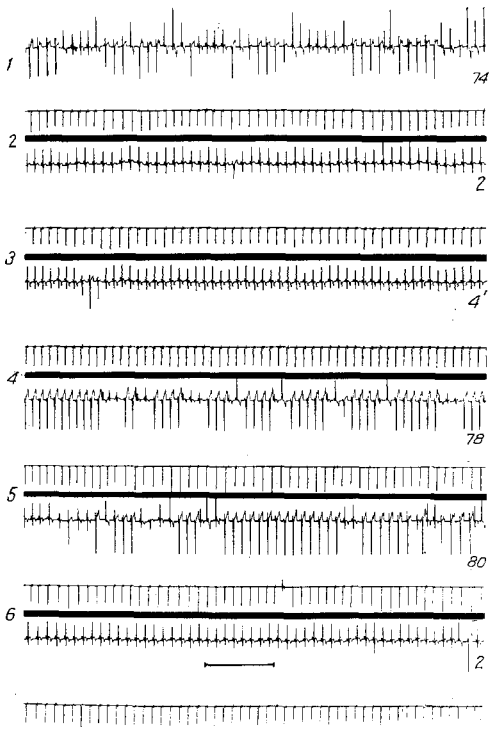

Fig. 1. Potentiation of antiarrhythmic action of lidocaine by tetrodotoxin. 1) 24 h after occlusion of coronary artery; 2 and 4) action of 4 and 2 mg/kg, respectively, of lidocaine for 4 min; 3 and 5) action of 2 and 1 μg/kg, respectively, of TTX for 3 min; 6) action of a mixture of 2 mg/kg lidocaine and 1 μg/kg TTX for 3 min. All fragments show ECG (top trace) and electrogram from auricle of right atrium (bottom trace). Number of ventricular extrasystoles per minute shown on right. Time marker 5 sec.

EXPERIMENTAL RESULTS

The results of one typical experiment are illustrated in Fig. 1 and they show that 24 h after occlusion of the coronary artery marked ventricular extrasystoles developed (asynchronism in excitation of atria and ventricles can be seen in fragment 1). TTX in a dose of 2 μg/kg and lidocaine in a dose of 4 mg/kg exhibited a marked antiarrhythmic action (fragments 2 and 3). In half these doses, these drugs had no effect on the arrhythmias (fragments 4 and 5). Injection of a mixture of subliminal doses of TTX and lidocaine caused virtually complete restoration of the sinus rhythm (fragment 6). The results of six experiments in which separate threshold doses of TTX and lidocaine and combined subliminal doses of both were injected.

The results show that TTX potentiates the antiarrhythmic action of lidocaine in the late stage of experimental myocardial infarction. Similar results were obtained previously with the combined action of etmosine and TTX and of mexiletine with TTX [2]. Potentiation by TTX of the antiarrhythmic action of lidocaine, etmosine, and mexiletine suggests that the cause of abolition of the arrhythmias in the late stage of experimental infarction under the influence of these drugs is a decrease in the fast inward sodium current. This conclusion is confirmed by additional observations: 1) TTX and etmosine abolish arrhythmias of the heart isolated 24 h after occlusion of the coronary artery [3]; 2) TTX and lidocaine block conduction of excitation in myocardial fragments isolated from the zone of ischemia [12]; 3) TTX, and also lidocaine and etmosine, in concentrations giving an antiarrhythmic action, selectively delay conduction in the zone of an infarct but have practically no effect on the conduction of excitation in the intact myocardium [4, 9, 10].

The mechanism of the antiarrhythmic action of drugs not only explains the cause of correction of the arrhythmias, but also indicates the possible causes of their actual development. The view is nowadays widely

TABLE 1. Antiarrhythmic Action of Threshold and a Mixture of Subliminal Doses of Lidocaine and TTX

Animal No.	Preparation and dose	Control		Maximal effect		Time, min†	Duration, min‡
		general rhythm, beats/min	VES, %*	general rhythm, beats/min	VES, %		
	Lidocaine, mg/kg						
1	4	119	50	118	0	1	15
2	12	126	100	102	44	2	8
3	12	184	98	138	0	2	20
4	4	150	98	130	0	7	18
5	4	132	77	116	21	1	5
6	4	100	74	96	2	4	8
$M \pm m$	6,8±1,7	135,2±11.8	82,8±8	116,7±6,5	11,2±7,4	2,8±0,9	12,3±2,5
	TTX, μg/kg						
1	2	136	60	125	0	3	27
2	3	136	95	126	0	3	19
3	6	182	98	140	0	6	11
4	3	148	81	130	31	7	19
5	1	170	93	142	8	1	10
6	2	98	79	96	4	3	8
$M \pm m$	2,8±07	145±12	84,3±5,8	126,5±6,8	7,2±4,9	3,8±0,9	15,7±3
	Lidocaine(mg/kg) + TTX (μg/kg):						
1	2+1	122	67	120	0	4	8
2	6+1,5	132	94	126	0	1	13
3	6+3	200	97	136	0	2	10
4	2+1,5	140	73	120	0	2	19
5	2+0,5	150	85	128	15	3	10
6	2+1	98	82	96	4	3	10
$M \pm m$	3,4±0,8 1,4±0,3	140,3±14	83±4,8	121±5,6	3,2±2,5	2,5±0,4	11,7±1,6

*Number of ventricular extrasystoles as a percentage of general cardiac frequency.
†Time taken to reach maximal effect after end of injection of drug.
‡Time taken to reduce antiarrhythmic effect by 50%.
Legend. Same number refers to same animal; decrease in percentage of VES significant ($P < 0.001$).

held that an increase in automatic activity of the Purkinje fibers in the late stage of myocardial infarction leads to the appearance of ectopic foci of excitation and to disturbance of the rhythm [11]. Automatic activity of Purkinje fibers is due to the pacemaker current I_{k_2}, which is depressed by lidocaine and by other antiarrhythmic agents [13]. That is why it has hitherto been possible to link together the mechanism of development and also the mechanism of suppression of arrhythmias in the late stage of infarction by means of drugs. However, these views are difficult to reconcile with observations showing the decisive role of a decrease in the inward sodium current in the production of the antiarrhythmic action of drugs. TTX, which has high antiarrhythmic activity [1, 3], does not affect the pacemaker current I_{k_2} of Purkinje fibers [6]. It can accordingly be postulated that if disturbances of rhythm in the late stage of infarction are in fact due to automatic activity, this must be bound with the sodium current. Automatic activity of this type arises in myocardial fibers and Purkinje fibers under the influence of the alkaloid aconitine.

In voltage clamp experiments aconitine has been shown to modify the properties of some fast sodium channels, and ultimately this leads to oscillations of membrane current and potential and gives rise to generation of repeated excitation [5]. Most antiarrhythmic drugs and TTX inhibit both aconitine-induced arrhythmias and arrhythmias arising in the late stage of infarction. Hence the hypothesis that the mechanism of disturbances of rhythm in the late stage of experimental myocardial infarction has common features with the mechanism of arrhythmias arising under the influence of aconitine. The analogy between the aconitine ectopic focus and ectopic activity developing 24 h after occlusion of the coronary artery emphasizes once again the importance of the fast sodium current in the development and termination of arrhythmias in the late stage of myocardial infarction.

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CHARACTER OF CORRELATION BETWEEN THE ANTICONVULSANT ACTION OF PHENAZEPAM AND ITS LEVEL IN THE MOUSE BRAIN

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Inhibition of the convulsant action of metrazol by injection of 1,4-benzodiazepine derivatives into experimental animals is a sensitive method which can be used for screening tranquilizers. By recording minimal effective doses of metrazol causing clonico-tonic convulsions and tonic extension against the background of injection of benzodiazepines it is possible to study quantitatively, in both alternative and graduated forms, the temporal dynamics of the anticonvulsant action of a test compound. In this case it is also possible to study the character of interaction between agonist (metrazol) and antagonist (benzodiazepine) in the formation of the pharmacological response by the agonist.

A method of simultaneous recording of the concentration of phenazepam-^{14}C and its metabolites in the mouse brain and the minimal effective doses of metrazol, intravenous injection of which causes tonic extension in animals, is suggested below. Correlation between these parameters is examined.

EXPERIMENTAL METHOD

Experiments were carried out on female (Black × BALB/c)F_1 mice weighing 18-20 g. Phenazepam-^{14}C (4 Ci/mmole) was injected in Tween emulsion intraperitoneally into the animals in a dose of 1.4 mg/kg (40 μmoles/kg). In the interval from 10 to 120 min after injection of the drug the minimal effective doses of metrazol causing tonic extension were determined in the animals [1]. Immediately after recording of the convulsant action of metrazol the animals were decapitated, the brain (0.36-0.48 g) was removed, and the radioactive material was extracted. For this purpose, the animals' brains were ground with anhydrous Na_2SO_4 (1 : 5 by weight) to form a dry powder, which was extracted with 7 ml chloroform. Model experiments showed that extraction 3 times was sufficient to extract all the radioactive material from the mouse brain. The pooled extracts were then evaporated in a vacuum drying cupboard at 50°C. The dry residue was dissolved in toluene–alcohol scintillator (10 ml) and the quantity of radioactive material was determined on an SL-30 liquid photometer (France). The results were subjected to statistical analysis [2].

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