Antiarrhythmic Effects of μ-Opiate Receptor Agonists in Rats with Epinephrine-Induced Arrhythmias: Role of the Vegetative Nervous System

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The μ-opiate receptor agonists DAGO and DALDA preinjected intravenously in a dose of 0.1 mg/kg prevented the occurrence of arrhythmias in rats injected with epinephrine. Their antiarrhythmic activity was not modified by atropine or hexamethonium. It is concluded that the vegetative nervous system plays no substantial role in mediating the antiarrhythmic effects of DAGO and DALDA, and that these effects are most likely associated with stimulation of cardiac opiate receptors.

Key Words: *μ-receptor ligands; arrhythmia; vagus*

Recent experimental studies have clearly demonstrated important contributions of the opioidergic system both to arrhythmogenesis and to the prevention of arrhythmias [2,5,9]. We found [2,5] that the enzyme-resistant μ- and δ-receptor agonist dalargin (D-Ala^2-Leu^5-Arg^6-Enkephalin) exhibits antiarrhythmic activity when injected intravenously and suggested [2] that peripheral opioid receptors (OR) may be involved in mediating the antiarrhythmic effects of this compound since the permeability of the blood-brain barrier for opioid peptides (OP) is low [7]. However, no direct evidence in support of this hypothesis was provided and, moreover, the mechanism and receptor specificity of the antiarrhythmic action exerted by dalargin upon systemic administration were not ascertained.

According to well-established views, activation of the sympathetic nervous system is conducive to ventricular tachyarrhythmias [8], whereas elevation of vagal tone increases electrical stability of the heart [4]. In addition, the vagus was shown to be reflexly stimulated by intravenously administered OP [12]. On this basis, we associated the antiarrhythmic effects of opioids with their modulating action on the vegetative nervous system [2].

The aim of our present study was to evaluate, on a rat model of epinephrine-induced arrhythmia, the role the vegetative nervous system may play in mediating the antiarrhythmic effects of selective μ-OR ligands.

MATERIALS AND METHODS

The study was conducted on male Wistar rats (body weight 150-200 g) in which arrhythmias were produced by epinephrine injected intravenously (100 μg/kg) under light ether anesthesia and the ECG was recorded in the second standard lead during the first 5 minutes postinjection. The endpoints considered in analyzing the ECGs were the incidence rates of ventricular extrasystoles, ventricular tachycardia, and ventricular fibrillation.

OR ligands were dissolved *ex tempore* in isotonic NaCl solution and injected intravenously at 15 min before epinephrine injection. The selective μ-OR agonists used were [D-Arg^2,Lys^4]dermorphin-(1-4)-amide (DALDA) [10] (synthesized by Professor P.W. Schiller from the Clinical Research Institute of Montreal, Canada) and [D-Ala^2,N-Me-Phe^4,Gly^5-

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TABLE 1. Effects of Opiate Receptor Ligands, Atropine, and Hexamethonium on Epinephrine-Induced Arrhythmias

Treatment	Number of rats	Without VA		VE		VT		VF	
		n	%	*n*	%	*n*	%	*n*	%
None (control)	34	3	9	14	41	15	44	2	6
DAGO	18	11^{++}	61	-$^{+}$	-	7	39	-	-
DALDA	32	22^{+++}	69	2^{++}	6	4^{+}	13	4	13
DAGO+naloxone	19	1***	5	9***	47	14*	73	1	5
DALDA+naloxone	20	2***	10	9***	45	14***	70	-	-
Naloxone	20	2	10	8	40	10	50	2	10
DAGO+atropine	23	13	57	2	9	9	39	-	-
DALDA+atropine	23	14	61	6*	26	6	26	1	4
Atropine	20	2	10	10	50	10	50	4	20
DAGO+hexamethonium	23	13	57	3	13	8	34	-	-
DALDA+hexamethonium	20	12	60	2	10	5	25	2	10
Hexamethonium	19	4	21	2^{+}	10	12	63	1	5

Note. $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ relative to the control group; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ relative to the group given DAGO or DALDA (as estimated by chi-squared test). VA = ventricular arrhythmia; VE = ventricular extrasystoles; VT = ventricular tachycardia; VF = ventricular fibrillation.

ol]-Enkephalin (DAGO) [3] (produced by BioPro Co., Novosibirsk). These peptides were each administered in a dose of 0.1 mg/kg, selected on the basis of our previously published data regarding the antiarrhythmic activity of dalargin [2,5]. As the μ-OR blocker, naloxone (Sigma) was used, injected intravenously in a dose of 0.2 mg/kg at 15 min before DALDA or DAGO [6]. To evaluate the contribution of the vegetative nervous system to the DALDA and DAGO effects, some rats were injected with atropine (1 mg/kg intravenously at 15 min before DALDA or DAGO) or, to produce "chemical denervation" of the myocardium, with hexamethonium (10 mg/kg intravenously at 15 min before DALDA or DAGO [1]).

The results were statistically analyzed by a chi-square test.

RESULTS

As shown in Table 1, only 3 of the 34 intact rats showed no ventricular arrhythmias after epinephrine injection; among the remaining rats, ventricular extrasystoles, tachycardias, fibrillations, or their combinations were recorded.

Preinjection with the peripheral μ-OR agonist DALDA increased 7-fold the percentage of animals without ventricular arrhythmia after epinephrine administration, while decreasing 6-fold the incidence of ventricular extrasystoles and 4-fold that of ventricular tachycardia. Very similar results were obtained with DAGO (Table 1).

Naloxone injected at 0.2 mg/kg, a dose sufficient to block only the μ-OR [6], was found to completely abolish the antiarrhythmic effects of both peptides — a finding arguing for participation of μ-OR in the mechanism of the antiarrhythmic action of DAGO and DALDA. Naloxone itself had no effect on the pattern of epinephrine-induced arrhythmias in the dose used.

Since permeability of the blood-brain barrier for OP is low [7], we assumed that the antiarrhythmic actions of DALDA and DAGO are associated with activation of peripheral μ-OR. Such activation is particularly likely in the case of DALDA, for this peptide cannot penetrate into the brain on systemic administration in doses less than 24 mg/kg [10].

The blockade of M-cholinergic receptors by atropine did not affect the antiarrhythmic activity of DAGO (Table 1) but weakened, though not eliminated, that of DALDA. Like naloxone, atropine injected alone before epinephrine had no effect on the pattern of the arrhythmias induced by the latter.

The interruption of vegetative nerve supply to the heart by the intravenously injected ganglion blocker hexamethonium [1] did not modify the antiarrhythmic effects of the μ-OR agonists. It should be noted that although, as shown in the Table 1, hexamethonium reduced 4-fold the proportion of rats with ventricular extrasystoles when administered alone, the statistical significance of this reduction was relatively low ($p<0.05$) and, unlike the opioids, hexamethonium failed to influence the incidence of ventricular tachycardia or fibrillation.

The results of the present study led us to conclude that the vegetative nervous system plays no substantial role in mediating the antiarrhythmic effects of DAGO and DALDA. The failure of atropine to

cancel DALDA's action indicates that the contribution of the vagal component to the antiarrhythmic effects of DALDA and DAGO is very small.

What, then, is the mechanism by which DAGO and DALDA act in preventing arrhythmias? At present, our answer to that question must of necessity be largely based on speculation. Since there is well-grounded evidence that of key importance in the genesis of epinephrine-induced arrhythmias are adenylate cyclase and an increase in the intracellular pool of cAMP [8] and since, on the other hand, cardiac myocyte membranes have been reported to carry receptors for OR [11], these receptors are probably coupled to, and capable of inhibiting, adenylate cyclase. Although no confirmatory evidence is available as yet, this possibility appears to be a very likely one, for, as our studies have indicated, enkephalins are able to prevent cardiac damage by Isadrin (isoproterenol) and lower the cAMP level in the myocardium [2,5].

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