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Effects of U-50 488H, a κ-agonist, on action potentials of isolated ventricular papillary muscle of guinea pigs[1]

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ABSTRACT Trans(+—)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl) cyclohexyl]-benzeneacetamide-methanesulfonate-hydrate (U-50 488H), a specific κ-agonist, at 1—10 μmol·L^{-1} caused concentration-dependent reductions in the action potential duration at 50% and 90% of repolarization (APD_{50} and APD_{90}) without modifying the resting potential (RP), the action potential amplitude (APA) and the maximal upstroke velocity (V_{max}). The effects were attenuated by (—)-(1R,5R,9R)-5,9-diethyl-2-(3-furylmethyl)-2L-hydroxy-6,7-benzomorphan (Mr 2266 BS, 1 μmol·L^{-1}), a specific κ-antagonist which itself had no effect on the action potentials of the ventricular papillary muscle of guinea pigs, indicating that U-50 488H at 1—10 μmol·L^{-1} acts via specific cardiac κ-receptors. At 100 μmol·L^{-1}, U-50 488H not only shortened APD_{50} and APD_{90}, but also reduced RP, APA, and V_{max}, which were not attenuated by Mr 2266 BS (1 μmol·L^{-1}) suggesting that the effects of U-50 488H at 100 μmol·L^{-1} were probably non-specific.

KEY WORDS U-50 488H; Mr 2266 BS; endorphin receptors; action potentials; papillary muscles; guinea pigs

Trans-(+—)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl) cyclohexyl]-benzeneacetamide-methanesulfonate-hydrate (U-50 488H), a specific κ-agonist, induces arrhythmias in the isolated perfused rat hearts and the arrhythmogenic effect is attenuated by (—)-(1R,5R,9R)-5,9-diethyl-2-(3-furylmethyl)-2L-hydroxy-6,7-benzomorphan (Mr 2266 BS), a specific κ-antagonist[1-3]. However, it is not clear whether the effects are opioid receptor-mediated although it was found that U-50 488H at 10 μmol·L^{-1} affects action potentials, twitch contraction, and calcium influx in guinea pig ventricular myocytes[4]. In the present study, we investigated the effects of U-50 488H on action potentials of ventricular papillary muscle of guinea pigs and more importantly studied whether the effects were attenuated by a specific κ-antagonist, Mr 2266 BS in order to determine if the effect of U-50 488H is opioid receptor-mediated.

MATERIALS AND METHODS

Twelve guinea pigs (285±*s* 25 g) of both sex were stunned. The hearts were quickly placed in 4 Tyrode's solution. The right papillary muscle was s perfused with Tyrode's solution bubbled with 95 % + 5 % CO_2 at a rate of 8—10 ml·min^{-1} (34.5 + 0 ℃). Tyrode's solution was made of: NaCl 137, Na CO_3 11.9, NaH_2PO_4 0.44, $MgCl_2$ 0.5, $CaCl_2$ 1. KCl 4, and glucose 5 mol·L^{-1}, pH 7.4.

The muscle was electrically stimulated at 1 with rectangular pulses (0.1 ms in duration, 1.5 tim threshold) delivered through a bipolar silver electrod Transmembrane action potentials were recorded usi conventional microelectrode techniques. The acti potential was displayed on a storage oscilloscc (COS5020-ST). The variables measured were R APA, APD_{50}, APD_{90}, and V_{max} which was determin by an electronic differentiating circuit. The musc were allowed to equilibrate in Tyrode's solution for 1 2 h before exposure to drugs.

In one protocol, action potentials were record before or 20 min after cumulative addition U-50 488H 1, 10, 100 μmol·L^{-1}. In another pro col, action potentials were recorded when musc

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were perfused with Tyrode's solution containing Mr 2266 BS (1 μmol·L^{-1}) at 5 min before and after addition of U-50 488H.

U-50 488H and Mr 2266 BS were kindly supplied by Upjohn Co and Boehringer Ingelheim Co, respectively. All chemicals used were AR.

Data were obtained only from preparations in which continuous microelectrode impalements were maintained throughout the experiment. The data were analysed with paired Student's t test.

RESULTS

U-50 488H at 1—100 μmol·L^{-1} shortened both APD_{50} and APD_{90} in a dose dependent manner. At 100 μmol·L^{-1}, it also reduced RP, APD, and V_{max} (Tab 1 and Fig 1a). The effects of U-50 488H at 1—10 μmol·L^{-1} on APD were attenuated by Mr 2266 BS at 1 μmol·L^{-1} (Tab 1 and Fig 1b), which alone produced no significant change on the action potentials when superfused for up to 2 h (Data not shown). The effects of U-50 488H at 100 μmol·L^{-1} were not affected by Mr 2266 BS significantly (Tab 1 and Fig 1b).

DISCUSSION

The present study showed that U-50 488H led to a Mr 2266 BS-reversible shortening of APD of the ventricular papillary muscle of guinea pigs at 1—10 μmol·L^{-1}, which is in agreement with our previous findings that U-50 488H induces arrhythmias in the isolated perfused rat heart and that the arrhythmogenic effect is attenuated by Mr 2266 BS[3]. The results indicate that the effects of the drug at this dose range are via specific κ-opioid receptors. Although the shortening of APD does not always result in arrhythmia, our findings suggest that the arrhythmogenic effect of U-50 488H may be related to the shortening of APD[3,7]. However, the mechanism of action of U-50 488H on shortening of APD and its contribution to arrhythmia is not very clear.

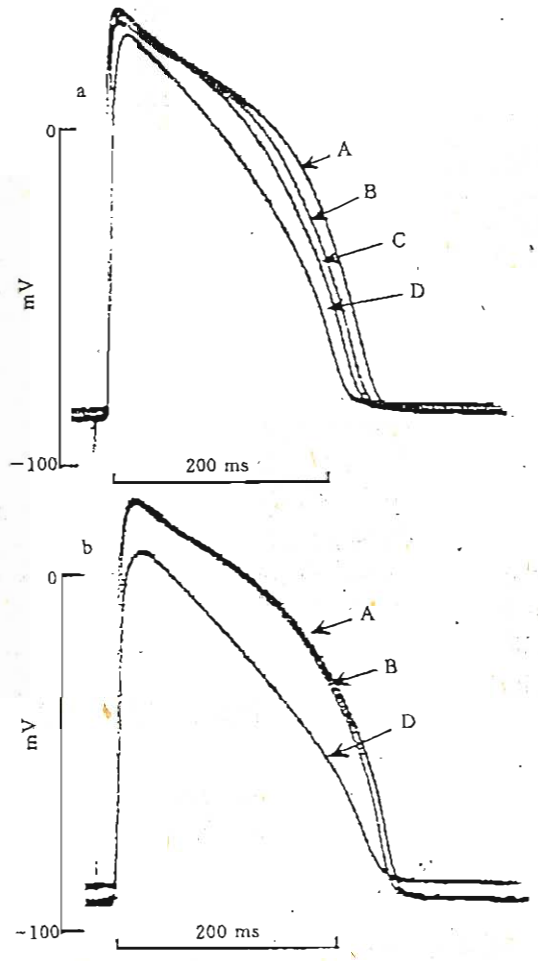

Fig 1. Effects of U-50 488H on action potentials of ventricular papillary muscle of guinea pigs in the absence (a) and the presence (b) of Mr 2266 BS at 1 μmol·L^{-1}. Concentration of U-50 488H (μmol·L^{-1}): A: 0; B: 1; C: 10; D: 100. The action potential profile in the presence of Mr 2266 BS at 1 μmol·L^{-1} was the same as that in the absence of the antagonist (not shown). Driving rate: 1 Hz.

At the concentration of 100 μmol·L^{-1}, U-50 488H not only shortened APD, but also RP, APA, and V_{max} and the effects were not antagonized by Mr 2266 BS. The result is in agreement with the observations by duBell and Lakatta[4] although they did not determine if the effects of U-50 488H were antagonized by

Tab 1. Effects of U-50 488H (μmol·L^{-1}) on action potentials of ventricular papillary muscle of guinea pigs in t absence (n = 8) and the presence (n = 4) of Mr 2266 BS at 1 μmol·L^{-1} $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.0$ *vs* 0.

	In the absence of Mr 2266 BS				In the presence of Mr 2266 BS			
U-50 488H	0	1	10	100	0	1	10	100
RP/mV	−91±2	−91±2^a	−91±3^a	−88±3^b	−90±2	−90±3^a	−90±1^a	−87±1^b
APA/mV	134±4	134±8^a	133±8^a	123±8^c	130±3	130±1^a	128±5^a	117±2^c
V_{max}/V·s^{-1}	200±10	200±1^a	199±11^a	178±14^c	200±3	200±3^a	199±3^a	159±11^c
APD_{50}/ms	196±12	181±16^c	174±17^c	152±20^c	186±15	188±19^a	184±17^a	156±6^c
APD_{90}/ms	240±17	226±20^b	221±20^c	207±23^c	232±14	236±16^a	234±15^a	210±3^b

opioid antagonists. In our previous[5,6] and present studies, higher concentrations of U-50488H produce effects not antagonized by Mr 2266 BS, suggesting that suggesting that the effects of the κ-agonist may be most likely non-specific. In conclusion the present study showed the κ-receptor-mediated and non-specific effects of U-50 488H, a specific κ-agonist, on action potential of the guinea pig papillary muscles. Further and more thorough studies are needed to unveil the ionic basis of the effects resulted from activation of cardiac κ-opioid receptors although it has been shown that U-50488H decreases intracellular free calcium transient in myocytes stimulated electrically[8].

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