Positive Inotropic Action of \(\kappa\) Opiate Agonists, Ethylketocyclazocine and Dynorphin-A(1-13), in Isolated Rat Atrium

Sadaaki MAEDA, Junji NAKAMAE and Reizo INOKI
Department of Pharmacology, Osaka University, Faculty of Dentistry, Yamadaoka 1-8, Suita, Osaka 565, Japan

Accepted July 4, 1987

Abstract—The effect of various opiate agonists on the contraction of isolated rat atrium was investigated. Ethylketocyclazocine (EKC) (30–100 \(\mu\)M) and dynorphin-A(1–13) (10–30 \(\mu\)M), which are \(\kappa\)-type agonists, caused positive inotropic effects on electrically stimulated left atrium in a dose-dependent manner. In addition, EKC decreased the frequency of spontaneous beating in the right atrium. Morphine (\(\mu\)-type), [Met\(^5\)] and [Leu\(^5\)]-enkephalin (\(\delta\)-type) did not affect both the developed tension and frequency of contractions. These results indicate that the positive inotropic action is specific for \(\kappa\)-type opiate agonists.

Most of the cardiovascular effects of opioids seem to be mediated by the central nervous system (1, 2). However, recent studies suggest that opioids can directly affect on cardiovascular system (3, 4). The existence of dynorphin, [Met\(^5\)]- and [Leu\(^5\)]-enkephalin in the heart has been shown by using bioassay and radioimmunoassay (5–7). Pharmacological and biochemical studies also indicate that opiate receptors may be present in the heart (8, 9). We have found that \(\kappa\)-type opiate agonists, EKC and dynorphin-A(1–13), markedly inhibit the activity of Na\(^+\),K\(^+\)-ATPase prepared from rat heart (S. Maeda et al., unpublished observation). Therefore, in order to investigate the direct action of \(\kappa\)-type opiate agonists on cardiac function, we studied here the effects of EKC and dynorphin-A(1–13) on spontaneously beating right atrium and electrically stimulated left atrium isolated from rat heart.

Male Sprague-Dawley rats, weighing 250–300 g, were sacrificed by a sharp blow on the head, and the hearts were quickly removed. The right and left atria were excised and vertically suspended in chambers containing 5 ml of Krebs-Henseleit bicarbonate buffer solution (in mM: NaCl, 118; NaHCO\(_3\), 27; KCl, 4.8; KH\(_2\)PO\(_4\), 1; CaCl\(_2\), 1.2; MgCl\(_2\) 1.2; and glucose, 11.1). The solution was constantly aerated with a 95% O\(_2\)-5% CO\(_2\) gas mixture at 30°C, yielding a final pH of 7.4. The right atrium was allowed to beat spontaneously, and left atrium was electrically stimulated at 1.5 Hz with square wave pulses of 4 msec duration at a voltage approximately 20% above the threshold, using platinum electrodes for field stimulation. The resting tension of either the right or left atrium was adjusted to 1.0 g, and changes in developed tension were monitored continuously using a force-displacement transducer (Nihon Kohden RM-150) and a polygraph recorder (Nihon Kohden TB-611T). The frequency of spontaneous contractions of right atrium was estimated by counting the number of contractions per minute. Each preparation was equilibrated for 60 min and then exposed to opiate agonists. In some experiments, naloxone, propranolol or verapamil was added to the incubation medium 30 min before the addition of opiate agonists.

When the spontaneously beating right atrium of rat heart was exposed to EKC (10–50 \(\mu\)M), both a marked increase in developed tension and a modest reduction in the frequency were observed (Fig. 1). Thirty \(\mu\)M dynorphin-A(1–13) caused a small increase in developed tension, but did not affect the frequency of spontaneous contractions in the
right atrium (data not shown). It has been reported that a decrease in frequency of contractions can produce an increase in developed tension in rat heart (10, 11). Therefore, in order to assess precisely the positive inotropic action of EKC and dynorphin-A(1-13), effects of these \(\kappa\)-type agonists were examined in electrically stimulated left atrium. The addition of EKC (30-100 \(\mu\)M) and dynorphin-A(1-13) (10-30 \(\mu\)M) caused an increase in the developed tension in a dose-dependent manner (Fig. 2). Morphine (\(\mu\)-type), [Met\(^5\)]-enkephalin and [Leu\(^5\)]-enkephalin (\(\delta\)-type) did not significantly alter the frequency and developed tension of both atria even at the concentration of 100 \(\mu\)M. These findings indicate that the inotropic action on isolated rat atrium is specific for \(\kappa\)-type agonists. Five \(\mu\)M propranolol or 0.2 \(\mu\)M verapamil, which by itself caused a significant decrease in the developed tension, failed to affect the positive inotropic action of 50 \(\mu\)M EKC and 30 \(\mu\)M dynorphin-A(1-13) (data not shown). This result may suggest that the positive inotropic action of EKC and dynorphin-A(1-13) is not mediated by the stimulation of adrenergic receptors or is not involved in changes of \(\text{Ca}^{2+}\) channels. In addition, naloxone (1-100 \(\mu\)M) also failed to affect the positive inotropic action of these drugs. Jacquet reported that morphine had an excitatory action in the vas deferens which was mediated by a naloxone-insensitive and non-stereospecific receptor (12). It was also reported that [Met\(^5\)]-enkephalin facilitated the spontaneous electrical and mechanical activity on the rat portal vein and the effect was not antagonized by naloxone (13). These observations suggest that the inotropic action of EKC and dynorphin-A(1-13) may be mediated by a naloxone-insensitive opiate receptor. Our recent study has revealed that EKC and dynorphin-A(1-13) markedly inhibit the activity of Na\(^{+}\), K\(^{+}\)-ATPase prepared from rat heart, which is not blocked by naloxone, and other type agonists have no effect (S. Maeda et al., unpublished observation). Thus, the positive inotropic action of EKC and dynorphin-A(1-13) observed in this study may be mediated by the inhibition of cardiac Na\(^{+}\), K\(^{+}\)-ATPase. However, much more work is necessary to determine the mechanism of the inotropic effect of EKC and dynorphin-A(1-13). The present study demonstrates that \(\kappa\)-opiate agonists, EKC and dynorphin-A(1-13), have a positive inotropic action.
Fig. 2. Inotropic effect of EKC and dynorphin-A(1–13) in electrically stimulated left atrium of rat heart. Left atrium was electrically stimulated at 1.5 Hz. After a 60 min equilibration (time zero), EKC (○, 30 μM; ●, 50 μM; △, 100 μM) or dynorphin-A(1–13) (□, 10 μM; ●, 30 μM) was added to the incubation medium. Closed square represents the developed tension of the control (no addition). Each point represents the mean of four to eight separate experiments. Vertical lines indicate S.E.

which is not mediated by the central nervous system.

Acknowledgment: This work has been supported in part by a Grant-in-Aid for Scientific Research (No. 60770163) from the Ministry of Education, Science and Culture, Japan.

References
1 Holaday, J.W.: Cardiovascular effects of endogenous opiate systems. Annu. Rev. Pharmacol. Toxicol. 23, 641–694 (1983)
2 Hassen, A.H., Feuerstein, G. and Faden, A.I.: Differential cardiovascular effects mediated by mu and kappa opiate receptors in hindbrain nuclei. Peptides 4, 621–625 (1983)
3 Ledda, F. and Mantelli, L.: Possible presynaptic inhibitory effect of etorphine on sympathetic nerve terminals of guinea-pig heart. Eur. J. Pharmacol. 85, 247–250 (1982)
4 Wong-Dusting, H.K. and Rand, M.J.: Effect of [D-Ala², Met⁶]-enkephalinamide and [D-Ala², D-Leu⁵]enkephalin on cholinergic and noradrenergic neurotransmission in isolated atria. Eur. J. Pharmacol. 111, 65–72 (1985)
5 Hughes, J., Kosterlitz, H.W. and Smith, T.W.: The distribution of methionine-enkephalin and leucine-enkephalin in the brain and peripheral tissues. Br. J. Pharmacol. 61, 639–647 (1977)
6 Lang, R.E., Herman, K., Dietz, R., Gaida, W., Ganten, D., Kraft, K. and Unger, T.: Evidence for the presence of enkephalins in the heart. Life
7 Spampinato, S. and Goldstein, A.: Immunoreactive dynorphin in rat tissues and plasma. Neuropeptides 3, 193–212 (1983)

8 Saxon, M.E., Ivanitsky, G.R., Beloyartsev, F.F., Safronova, V.G., Kokoz, Yu. M. and Freydin, A.A.: Myocardial opiate receptors. Gen. Physiol. Biophys. 1, 447–452 (1982)

9 Ruth, J.A. and Eiden, L.E.: Leucine-enkephalin modulation of catecholamine positive chronotrophy in rat atria is receptor-specific and calcium-dependent. Neuropeptides 4, 101–108 (1984)

10 Langer, G.A., Brady, A.J., Tan, S.T. and Serena, S.D.: Correlation of the glycoside response, the force staircase, and the action potential configuration in the neonatal rat heart. Circ. Res. 36, 744–752 (1975)

11 Temma, K., Akera, T. and Brody, T.M.: Inotropic effects of digitoxin in isolated guinea-pig heart under conditions which alter contraction. Eur. J. Pharmacol. 76, 361–370 (1981)

12 Jacquet, Y.F.: Excitatory and inhibitory effects of opiate in rat vas deferens: A dual mechanism of opiate action. Science 210, 95–97 (1980)

13 Yamamoto, Y., Hotta, K. and Matsuda, T.: Effect of methionine-enkephalin on the spontaneous electrical and mechanical activity of the smooth muscle of the rat portal vein. Life Sci. 34, 993–999 (1984)