Effects of a New Cardiotonic Agent 1,2-Dihydro-6-methyl-2-oxo-5-[imidazo(1,2-a)pyridin-6-yl]-3-pyridine Carbonitrile Hydrochloride Monohydrate (E-1020) on Contractile Force and Cyclic AMP Metabolism in Canine Ventricular Muscle

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Abstract—E-1020, a newly synthesized imidazopyridinylpyridine or imidazopyridine derivative (structurally closely related to the bipyridine derivative milrinone) increased the force of contraction and cyclic AMP levels in a concentration-dependent manner in the isolated canine ventricular trabeculae electrically driven at 0.5 Hz at 37°C. The concentration-response curve for the increase in force of contraction by E-1020 was biphasic. The maximal positive inotropic effect (PIE) of E-1020 is comparable to that of milrinone, and its potency is 3-fold less than that of milrinone, but 10-fold higher than that of amrinone. The time course of increases in force of contraction induced by E-1020 was coincident with that of cyclic AMP accumulation. The concentration-response curve for the PIE of E-1020 was superimposable to that of cyclic AMP accumulation. A β-adrenoceptor antagonist, (+)-bupranolol (3×10⁻⁷ mol/l), did not affect the PIE of E-1020. The increase in the force of contraction and accumulation of cyclic AMP produced by E-1020 were inhibited by a muscarinic receptor agonist, carbachol. The relationship between the force of contraction and cyclic AMP levels in the presence of E-1020 was not modified by addition of carbachol or isoproterenol. E-1020 shifted the concentration-response curve for isoproterenol to the left. E-1020 shortened the total duration of contraction and relaxation time of isometric contractions. These findings indicate that cyclic AMP is essentially involved in the PIE of E-1020 on the canine ventricular muscle, although the possible involvement of a cyclic AMP-independent mechanism can not be excluded.

Currently, there has been an intensive effort to develop orally-active, cardiotonic and vasodilating agents with novel mechanisms of action for the treatment of congestive heart failure (1, 2). Bipyridine derivatives, amrinone and its congener milrinone, belong to the major class of such newly developed cardiotonic agents (1–4). The cellular mechanism of the positive inotropic effect (PIE) of bipyridine derivatives is not fully clarified and still remains controversial. Bipyridine derivatives have been shown to inhibit the cardiac phosphodiesterase (PDE), FIII fraction (cyclic AMP specific PDE with low Km, inhibited by cyclic GMP) and accumulate cyclic AMP in myocardial tissues (5–9). Therefore, the inhibition of the FIII fraction by bipyridine derivatives has been implicated to be an essential mechanism of the PIE of these compounds (10, 11). However, a closer inspection of the relation between the time course or concentration-dependence of PDE inhibition or accumulation of cyclic AMP and those of the increase in force of contraction during induction of the PIE by bipyridine derivatives revealed a definite apparent discrepancy in a number of experimental preparations (7, 9, 11). Furthermore, it has been found that the concentration-response curve (CRC) for the PIE of milrinone is biphasic (9, 11, 12) and
that milrinone induces biphasic contractions in the ferret ventricular muscle at low temperature (13), which are not explainable by accumulation of cyclic AMP resulting from inhibition of FIII-PDE. These findings led some authors to postulate that some subcellular mechanisms other than accumulation of cyclic AMP may be involved in the PIE of bipyridine derivatives (7, 11–13).

E-1020 [1,2-dihydro-6-methyl-2-oxo-5-(imidazo(1,2-a)pyridin-6-yl)-3-pyridine carbonitrile hydrochloride monohydrate] is a newly developed imidazopyridinylpyridine or imidazopyridine derivative cardiotonic agent with a vasodilating effect (Fig. 1). It has recently been shown that E-1020 causes the PIE with little increase in heart rate in the anesthetized dog (14). While there is good evidence that cyclic AMP may mediate the PIE of bipyridine derivatives, mechanisms other than cyclic AMP accumulation have likewise been postulated to be involved in the cardiotonic effects of amrinone (14) and milrinone (7, 11–13). Therefore, pharmacological analysis of the effects of E-1020, structurally closely related to the bipyridine derivative milrinone, may be helpful to provide insight into the mechanism involved in the PIE of E-1020 in relation to cyclic AMP metabolism in the isolated canine ventricular muscle.

**Materials and Methods**

Preparation and experimental procedures: The heart was excised from mongrel dogs of either sex, weighing 6 to 13 kg, anesthetized with sodium pentobarbital, 30 mg/kg, i.v. Thin trabeculae carneae (less than 1 mm in diameter and 6 to 9 mm in length) were isolated from the right ventricle in oxygenated cold (ca. 4°C) Tyrode solution, and mounted in 20-ml organ baths filled with Krebs-Henseleit solution (containing 0.057 mmol/l ascorbic acid and 0.027 mmol/l disodium EDTA in order to minimize the rate of autooxidation of catecholamines during the experiments). The composition of the solution was as follows: 118 mmol/l NaCl, 4.7 mmol/l KCl, 2.5 mmol/l CaCl₂, 1.2 mmol/l MgSO₄, 1.2 mmol/l KH₂PO₄, 24.9 mmol/l NaHCO₃ and 11.1 mmol/l glucose. The solution was equilibrated by bubbling with 95% O₂ and 5% CO₂ at a temperature of 37°C (pH 7.4). Muscles were stretched to a resting tension of about 5 mN and stimulated by square wave pulses of a voltage of 20% above threshold and 5-msec duration at a frequency of 0.5 Hz. The force of the isometric contractions was measured by force displacement transducers (Shinkoh Communication Industry Co., Tokyo, UL-10GR) and recorded by a thermal-pen recorder (NEC San-ei Instrument Ltd., Tokyo, Recti-Horiz-8K). Four muscles isolated from one heart were run in parallel, and one of them always served as a control. (+)-Bupranolol, 3×10⁻⁷ mol/l, was used to examine whether or not the direct effect of E-1020 is modified by norepinephrine released from nerve endings by the compound. After the 1-hr equilibration period, (+)-bupranolol was added and allowed to act for 30 min before the administration of E-1020.

The time to peak force (TTP), relaxation time (RT) and total duration of contraction (TDC) of single contractions were analyzed by a data processor (Nihon Kohden, ATAC 450). The amplified signal from each force transducer was fed to one of four inputs of the AD converter part (10 bit and sampling time: 1 or 2 msec) of the data processor and was digitized and stored. Software was developed to continuously analyze the contractile parameters. Data could be displayed immediately on a cathode ray tube and printed out by a printer (Ricoh, Tokyo, SD 120).

In some experiments, ventricular muscles were removed from the bath at various times after the administration of E-1020 and immediately frozen in liquid nitrogen. Frozen
muscles were stored overnight at \(-80^\circ\text{C}\) until homogenized. Cyclic AMP contents of the frozen muscle samples were determined as described previously (6), and the effects of E-1020 on the cyclic AMP levels of the tissues in relation to the strength of isometric contractions were assessed as time- and concentration-dependent responses.

**Drugs and chemicals:** Drugs and chemicals used were as follows: E-1020 \([1,2\text{-dihydro-6-methyl-2-oxo-5-(imidazo(1,2-a)pyridin-6-yl)-3-pyridine carbonitrile hydrochloride monohydrate}]\) (Eisai Co. Ltd., Tokyo), (-)-isoproterenol hydrochloride (Sigma Chemical Co., St. Louis, MO), carbachol (carbamylcholine chloride) (K & K Laboratories, New York, NY), (±)-bupranolol hydrochloride (Kaken Kagaku Co., Tokyo). The antisera to cyclic AMP and [125I]-succinyl cyclic AMP tyrosine methylester were prepared and supplied by Yamasa Shoyu Co. (Choshi, Chiba, Japan).

**Statistical analysis:** Experimental values were presented as the mean±S.E.M. The difference between mean values was evaluated by Student’s \(t\)-test. \(P\) values smaller than 0.05 were considered to represent significant differences.

**Results**

**Inotropic effects of E-1020 and influence of (±)-bupranolol:** The CRC for E-1020 on force of contraction in isolated canine right ventricular trabeculae is shown in Fig. 2. E-1020 caused the PIE in a concentration-dependent manner at \(3\times10^{-7}\) mol/l and higher. The CRC of E-1020 appears to be biphasic: the first component reached a plateau at \(3\times10^{-5}\) mol/l and the second steeper component was observed at \(10^{-4}\) mol/l and higher (this is observed more clearly in Fig. 6). The EC50 and maximal response to E-1020 were \(3.1\times10^{-6}\) mol/l and 0.51 (the maximal response to isoproterenol was taken as 1.0). (±)-Bupranolol did not affect both the components of the PIE of E-1020. The EC50 and maximal response to E-1020 in the presence of (±)-bupranolol were \(3.2\times10^{-5}\) mol/l and 0.42, respectively, which were not significantly different from those in the absence of (±)-bupranolol.

E-1020 affected the time course of contraction relatively less. It shortened slightly but significantly the RT and TDC only at \(3\times10^{-4}\) mol/l, the highest concentration tested (Fig. 3).

**Effects of E-1020 on cyclic AMP levels:** The time course of changes in force of contraction and cyclic AMP levels in response to E-1020 \(10^{-4}\) mol/l is shown in Fig. 4. Cyclic AMP levels and the force of contraction were increased substantially in a similar time course after administration of \(10^{-4}\) mol/l E-1020. The force of contraction began to increase significantly at 1 min after the administration, and it reached the maximal level at 10 min. The increase in cyclic AMP levels reached a significant level at 5 min. The force of contraction and cyclic AMP levels decreased slightly at 20 min.

The CRC for E-1020 on the force of contraction and cyclic AMP levels determined at 10 min after the administration is presented in Fig. 5. The force of contraction increased...
Fig. 3. Effects of E-1020 administered in a cumulative manner on the time course of individual isometric contractions in the presence of 3×10^{-1} mol/l (±)-bupranolol. TDC: total duration of contraction, TTP: time to peak force, RT: relaxation time. Values were obtained from the same experiments as presented in Fig. 2. Vertical bars: ±S.E.M. Asterisks indicate the significant changes from the respective controls.

significantly with E-1020 at 3×10^{-6} mol/l and higher. Similarly, the increase in cyclic AMP levels reached a significant level at 10^{-5} mol/l E-1020 and higher. Thus, the cyclic AMP level and force of contraction increased with E-1020 substantially in a similar concentration range.

Influence of carbachol: Carbachol has been shown to inhibit specifically the cyclic AMP-mediated PIE of cardiotonic agents without affecting the PIE elicited through cyclic AMP-independent mechanisms such as those involving an elevation of extracellular calcium, g-strophanthin and α-adrenoceptor agonists in the canine and rabbit right ventricular muscle (15-17). We investigated, therefore, the influence of carbachol on the E-1020-induced effects in order to further elucidate the role of cyclic AMP in the PIE of E-1020. The CRC for E-1020 was shifted to the right and downward by carbachol in a concentration-dependent manner (Fig. 6). Carbachol, 3×10^{-6} mol/l, completely inhibited the first component of CRC for E-1020, while the second component was observed even in the presence of the maximally effective concentration of carbachol.

Influence of 3×10^{-6} mol/l carbachol on the increase in force of contraction and cyclic

Fig. 4. Time course of changes in force of contraction and cyclic AMP levels after administration of 10^{-4} mol/l E-1020 in the presence of (±)-bupranolol, 3×10^{-7} mol/l, in the canine right ventricular trabeculae. Each symbol represents mean values in five preparations (n=5). Asterisks indicate significant differences from the respective control values. Basal force before the administration of E-1020: 4.9±0.24 mN (n=5).
Fig. 5. The concentration-response curves for the effect of E-1020 on force of contraction and cyclic AMP levels in the presence of 3×10⁻⁷ mol/l (±)-bupranolol in the canine right ventricular trabeculae. All values were determined 10 min after the administration of E-1020. Each symbol represents mean values in five preparations (n=5). Asterisks indicate significant differences from the respective control values.

Fig. 6. Concentration-response curves for the positive inotropic effect of E-1020 on force of contraction in the absence or presence of 3×10⁻⁸, 3×10⁻⁷ and 3×10⁻⁶ mol/l carbachol in the canine right ventricular trabeculae. Numbers in parentheses: numbers of experiments. Vertical bars: ±S.E.M. Basal force and maximal response to isoproterenol: 3.1±0.62 and 8.0±2.07 mN (n=5, control), 2.9±0.72 and 9.2±2.98 mN (n=5, 3×10⁻⁸ mol/l carbachol), 3.2±1.68 and 11.0±3.69 mN (n=5, 3×10⁻⁷ mol/l carbachol), 3.5±1.70 and 12.9±4.92 mN (n=5, 3×10⁻⁶ mol/l carbachol). AMP levels caused by 10⁻⁴ mol/l E-1020 is shown in Fig. 7. Carbachol alone does not significantly affect the force of contraction and cyclic AMP levels (14, 15), but it markedly reduces the PIE (Fig. 7A and B) and the increase in cyclic AMP levels (Fig. 7B) induced by 10⁻⁴ mol/l E-1020.

Influence of E-1020 on the effects of isoproterenol: Influence of E-1020 on the CRC for the PIE of isoproterenol is shown in Fig. 8. The CRC for isoproterenol was shifted to the left and upward by E-1020 in a concentration-dependent manner. Since E-1020 increased the force of contraction by itself, the increase induced by E-1020 was subtracted from the total increase produced by E-1020 and isoproterenol, and the remaining increase caused by isoproterenol was expressed as 100%. The CRC for isoproterenol in the presence of 3×10⁻⁷, 10⁻⁶ and 3×10⁻⁶ mol/l E-1020 were 7.53±0.26 (n=5), 8.07±0.39 (n=5) and 8.35±0.13 (n=5), respectively, which were significantly higher than the control value of 6.84±0.06 (n=5). The cyclic AMP levels and force of
contraction determined during the interaction of E-1020 with isoproterenol are presented in Fig. 9. While the cyclic AMP level was elevated by isoproterenol plus E-1020 to a level significantly higher than those with the individual agents alone, the force of contraction in the presence of both agents was slightly but not significantly higher than that with isoproterenol alone.

Relationship between force of contraction and cyclic AMP levels in the presence of E-1020: Figure 10 shows the relationship between force of contraction and cyclic AMP levels in individual muscles after administration of E-1020 alone and E-1020 with carbachol or isoproterenol. All values determined after the administration of E-1020 were included for the calculation of correlation coefficients. A significant correlation between the force of contraction and cyclic AMP levels was found in the presence of E-1020 alone: the relation was $Y=0.00532X+0.569$, where $Y$ was the cyclic AMP levels in pmol/mg w.w., and $X$ was the force of contraction expressed as the percentage of basal force ($r=0.6141$, $n=65$, $P<0.001$). When the values determined in the presence of E-1020 plus carbachol and E-1020 plus isoproterenol were...
also included for calculation, the relation was not significantly different from that in the presence of E-1020 alone: \( Y = 0.00496X + 0.656 \) \((r=0.6033, n=77, P<0.001)\).  

\[ Y = 0.00496X + 0.656 \]

Discussion

E-1020 that is structurally closely related to milrinone elicited the PIE in a concentration-dependent manner in the canine right ventricular myocardium. The maximal response to E-1020 as a cardiotonic agent is comparable to that of milrinone, while E-1020 is about 3-fold less potent than milrinone in the same preparation (9). Thus, the order of effectiveness as cardiotonic agents is: milrinone > E-1020 > amrinone. As with other structurally related bipyridine derivatives (6, 9), the PIE of E-1020 was not affected by a potent \(\beta\)-adrenoceptor blocking agent, (±)-bupranolol, implying that the activation of \(\beta\)-adrenoceptors is not involved in the PIE of E-1020.

The following findings in the present study support the view that cyclic AMP is essentially involved in the PIE of E-1020, being consistent with recent findings in the isolated guinea pig cardiac muscle (18).

1) In the canine right ventricular muscle, the PIE of E-1020 was accompanied by a concomitant increase in cyclic AMP levels of the tissue. A close association of the increase in cyclic AMP levels with that in force of con-

![Graph](image)

**Fig. 8.** Influence of \(3\times10^{-7}, 10^{-6}\) and \(3\times10^{-6}\) mol/l E-1020 on the concentration-response curves for isoproterenol to increase the force of contraction in the canine right ventricular trabeculae. Numbers in parentheses: numbers of experiments. Vertical bars: ±S.E.M.

![Graph](image)

**Fig. 9.** Effects of \(10^{-7}\) mol/l isoproterenol, \(10^{-4}\) mol/l E-1020 and both on force of contraction and cyclic AMP levels in the canine right ventricular trabeculae. Cyclic AMP levels were determined 15 min after administration of E-1020, 10 min after isoproterenol, 10 min after isoproterenol in the presence of E-1020 (E-1020 was administered 5 min before isoproterenol). *P<0.05 vs. the respective control values, **P<0.01 vs. the value in the presence of E-1020 alone. Numbers in parentheses: numbers of experiments.
traction was found in the time- and concentration-response relationships for E-1020.

2) A muscarinic receptor agonist, carbachol, markedly suppressed the PIE and the increase in cyclic AMP levels produced by E-1020. Since the relationship between the force of contraction and cyclic AMP levels in the presence of E-1020 alone was not modified by carbachol, lowering of cyclic AMP levels by carbachol through activating $G_1$ to result in suppression of the catalytic subunit of adenylate cyclase (19, 20) may be responsible for muscarinic inhibition of the PIE of E-1020 in the canine ventricular muscle.

3) The PIE of isoproterenol was enhanced by E-1020 in a concentration-dependent manner, and the cyclic AMP level was higher in the presence of E-1020 plus isoproterenol than that with E-1020 or isoproterenol alone.

4) E-1020 ($3 \times 10^{-4}$ mol/l) significantly shortened RT, which is characteristic to cardiotonic agents such as $\beta$-adrenoceptor agonists, IBMX, milrinone and amrinone acting through an increase in the cyclic AMP level (21, 22).

These observations are in accordance with the previous findings that the PIE of bipyridine derivatives amrinone (5, 6) and milrinone (9) is primarily mediated by a cyclic AMP-dependent intracellular process. Nevertheless, there are some findings that require consideration with respect to the possible involvement of a mechanism unrelated to cyclic AMP accumulation.

An intriguing observation in the present experiments is that the CRC for E-1020 in the canine ventricular myocardium appears to be
biphasic (Figs. 2 and 6). The biphasic CRC has also reported in several preparations with milrinone (9, 11, 12, 23), and taken as potential evidence implying an additional mechanism of action of this compound. The present findings that the PIE of E-1020 at $10^{-4}$ mol/l and higher was rather resistant to the inhibitory action of carbachol is consistent with the view that a cyclic AMP-independent mechanism may partly be responsible for the PIE of E-1020. It has been shown that another bipyridine derivative milrinone does not increase the Ca$^{2+}$ sensitivity of myofibrillar Mg-ATPase (24). This implies that the increase in Ca$^{2+}$ sensitivity may be not responsible for the cyclic AMP-independent PIE of bipyridine derivatives; and therefore, the subcellular mechanism of the cyclic AMP-independent PIE of bipyridine derivatives reported in the previous (12, 13) and the present study is as yet unknown.

It has recently been shown that E-1020 inhibits selectively the PDE activity of the FIII fraction prepared from guinea pig heart with a slightly higher potency or one comparable to that of milrinone (18), and that the IC50 values for milrinone to inhibit the FIII fraction of guinea pig and canine cardiac muscle are identical (25). Thus the order of potency as cardiotonic agents is not coincident with that as inhibitors of the FIII fraction in vitro. Brunkhorst and co-workers (11) have described that the CRC of milrinone is biphasic, and the first component of the PIE of milrinone in lower concentrations correlates well to the ability of the compound to inhibit the FII fraction, but the second component hinders the examination of the correlation between the FIII inhibition and PIE. The difference in the extent of contribution of the cyclic AMP-dependent effect and cyclic AMP-independent one may play a role in the discrepancy of potencies for inhibition of the FIII fraction and PIE as suggested by Brunkhorst et al. (11). The cyclic AMP-independent mechanism may also contribute to the observation that the abbreviation of isometric contractions produced by E-1020 in the canine ventricular muscle is relatively less compared with those caused by amrinone (6) or milrinone (9) in the same preparation. It is noteworthy, however, that the cyclic AMP-independent mechanism appears to operate only at high concentrations of bipyridine derivatives including E-1020 even if the mechanism plays some roles in the regulation of myocardial contractility elicited by this class of cardiotonics.

In conclusion, the present results indicate that the newly synthesized cardiotonic agent E-1020, structurally closely related to the bipyridine derivative milrinone, may primarily increase myocardial contractility through accumulation of cyclic AMP, probably by inhibition of the FIII fraction of cardiac phosphodiesterase.

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