EFFECTS OF CATECHOLAMINES INJECTED INTO SINOATRIAL NODAL CELLS ON THEIR ELECTRICAL ACTIVITY

Yoshihide YAMASAKI, Motohatsu FUJIWARA and Noboru TODA

Department of Pharmacology, Faculty of Medicine, Kyoto University,
Sakyo-ku, Kyoto 606, Japan

Accepted October 16, 1973

Abstract Transmembrane potentials were recorded from single cells of the isolated rabbit sinoatrial node by microelectrodes filled with a mixture of isoproterenol or noradrenaline and KCl. The electrode was used for recording the membrane potential and also for the iontophoretic application of the amines. Intracellularly applied isoproterenol caused a significant increase in the slope of diastolic depolarization, resulting in tachycardia. However, the extent of these changes was markedly less than that obtained when the amine was extracellularly applied by the iontophoretic technique, and the latency for inducing the changes was longer. These effects were abolished by 10^-6 M propranolol applied to the bathing media. There were regional differences in the responsiveness of S-A nodal cells to intracellularly applied isoproterenol. An area from which true pacemaker action potentials were most often recorded, tended to be most sensitive to the amine. Noradrenaline applied intracellularly caused an increase in the slope of diastolic depolarization and a tachycardia only when the preparations were treated with 10^-6 M cocaine. These results suggest that adrenergic beta receptors are on the outside of the membrane of S-A nodal cells.

Evidence that acetylcholine elicits changes in the permeability of membranes for ions of skeletal and cardiac muscle cells only when acetylcholine is applied close to the membranes but not when injected into the cells (1, 2) suggests that cholinergic receptors are on the outside of cell membranes. The major change induced by catecholamines applied to the isolated heart soaked in the bathing media has been demonstrated to be an increase in the slope of diastolic depolarization in sinoatrial (S-A) nodal pacemaker cells, resulting in an acceleration of the heart rate (3). However, no information is yet available concerning the effect of intracellularly applied catecholamines and the comparison of the effects of intra- and extracellularly applied amines on the electrical activity of pacemaker cells.

Dominant pacemaker activity moves from one site to the other rich in sympathetic nerve supply, when the nerve is excited (4). Such a pacemaker shift has also been demonstrated upon the addition of adrenaline and noradrenaline to the bathing media (3, 4, 5), presumably due to a different sensitivity of S-A nodal cells to the amines. Pertinent information

1 Supported in part by the Scientific Research Fund (No. 848085) from the Ministry of Education of Japan. A preliminary report of this study has been presented (Japan. J. Pharmacol. 22: suppl. 26, 1972).
2 The data are taken from a dissertation which was submitted by Yoshihide Yamasaki to Kyoto University in partial fulfillment of the requirement for the degree of Doctor of Medical Science.
3 Present address: Department of Pharmacology, Kyoto Prefectural University of Medicine, Kamikyo-ku, Kyoto 602, Japan.
4 To whom requests for reprints should be addressed.
regarding the regional difference in sensitivity can be obtained only by examining the response to topically applied amines of single cells from different regions of the S-A node. The present study was undertaken to determine and compare effects of intra- and extracellularly applied isoproterenol and noradrenaline on the transmembrane potential of S-A nodal cells. The microiontophoretic technique was used for topically applying the amines. Regional differences in the responsiveness of S-A nodal cells to isoproterenol were also investigated.

MATERIALS AND METHODS

Albino rabbits of both sexes, weighing 1.8 to 2.4 kg, were benumbed by a blow on the neck and sacrificed by exsanguination from the common carotid arteries. The heart was removed and the right atrial preparation including the S-A node and the interatrial septum was prepared. The specimen was fixed horizontally, endocardial surface uppermost, between hooks under a resting tension of 100 to 300 mg in a muscle bath of 60 ml capacity containing the bathing fluid. Hooks anchoring an appendage of the atrium were connected to the lever arm of a force-displacement transducer (Nihonkoden Kogyo Co., Tokyo, Japan). The solution was bubbled with a gas mixture of 95% O₂ and 5% CO₂ through a glass filter and was maintained at 30 ± 0.5°C. The pH of the solution was 7.2 to 7.4. Constituents of the solution were as follows (mM): Na⁺, 162.1; K⁺, 5.6; Ca²⁺, 2.2; Cl⁻, 157.0; HCO₃⁻, 14.9; dextrose, 5.6. Preparations were allowed to equilibrate for 60 min in the bathing medium, before experiments were commenced.

Transmembrane potentials were recorded from single cells of the S-A node by glass microelectrodes with a resistance of 30 to 60 megohms. Electrodes were filled with 2.5 M KCl containing 0.001 to 0.1 M isoproterenol (isoproterenol electrode) or 0.1 to 1 M noradrenaline (noradrenaline electrode) and with 3 M KCl (KCl electrode). The pH of the solution was adjusted to 3 to 4. These electrodes were used for recording the membrane potential and also for iontophoretically applying the amines. The membrane potential was recorded from a VC-7 oscilloscope (Nihonkoden Kogyo Co.) on films and also on an ink-writing oscillograph (Sanei Sokki Co., Tokyo, Japan). The S-A nodal rate was taken as the mean value of ten measurements of the cycle length between action potentials or atrial contractions before the amine addition and also when the maximum response to the amine had been obtained.

In order to apply amines, the electrode was connected to a bridge circuit which was essentially the same as that described by Araki and Otani (6). As shown in Fig. 1, a high resistance (100 megohms) was placed in one of the arms to minimize fluctuations caused by iontophoretic currents. In the same arm, a galvanometer (GAL) which has an input impedance of 1 kilohms was placed to measure the current flowing through the circuit. To avoid deformity of action potentials due to high capacitance in the circuit, a switch was inserted between the galvanometer and 100 megohm resistor and was opened except when the current was applied. The time constant of a 100 mV pulse signal in this circuit was less than 0.5 msec when the switch was off. Electrical direct current was provided by an
In 4 preparations, double barrel electrodes were used. The KCl and the isoproterenol electrodes were fixed in parallel, the tip of the former protruding 20 to 40 μ beyond the other. The KCl electrode was connected directly to the preamplifier (AMP) whereas the isoproterenol electrode was placed as shown in Fig. 1 except that the connection to the preamplifier was left open. Indifferent electrodes each had their own recording and ejecting electrodes.

Drugs used were 1-isoproterenol hydrochloride, 1-noradrenaline hydrochloride, dl-propranolol hydrochloride and cocaine hydrochloride.

RESULTS

Effects of intracellularly applied isoproterenol

Intracellular application of isoproterenol by electrical current of 5 to 100 nA for a period of 5 to 60 sec caused an increase in the slope of diastolic depolarization in 49 of 80 S-A nodal cells from 43 preparations (Fig. 2). Increase in the slope was related directly to the current strength and the duration of current application in the same cells. In association with the increased slope of diastolic depolarization, the latent pacemaker was frequently converted to a true pacemaker (cf A and C in Fig. 3), which is characterized by smooth transition from diastolic depolarization to rapid upstroke (7). The increase in the slope of diastolic depolarization was usually accompanied by a tachycardia. However, when increase in the slope was not sufficient to depolarize membranes to the level of firing action potentials earlier, a hump was produced at the end diastole, as demonstrated in Fig. 3B. By increasing the current strength and the duration of current application, greater increase in the slope of diastolic depolarization was elicited; thus the hump was abolished and the electronic stimulator through an isolating unit (Nihonkoden Kogyo Co.).
FIG. 2. Effect of intracellularly applied isoproterenol on the membrane potential of a true pacemaker cell.

A) control; B) 300 sec after the start of isoproterenol injection (120 nA for 30 sec) Calibration: 50 mV, 1 sec.

FIG. 3. Effects of intracellularly applied isoproterenol on the membrane potential of a latent pacemaker cell.

A) control; B) 180 sec after injection of isoproterenol (120 nA for 30 sec); C) 90 sec after the second injection of isoproterenol (120 nA for 60 sec) All recording were obtained from the same cell. Note "hump" in B and conversion of the latent pacemaker cell to a true pacemaker in C. Calibration: 50 mV, 1 sec.
rate was accelerated (Fig. 3C). These effects of isoproterenol were abolished by treatment for 10 to 20 min with 10⁻⁶ M propranolol added to the bathing medium.

No significant change in the membrane potential was elicited by electrical current applied through a KCl electrode with the strength and the duration of application sufficient to cause the changes when the isoproterenol electrode was used. Even when the S-A nodal cells were penetrated for 3 to 5 min by the isoproterenol electrode, the electrical activity was not significantly altered without the current application.

Because of the different responsiveness of S-A nodal cells to intracellularly applied isoproterenol, responses were classified into 5 groups: (A) change neither in the membrane potential nor in the atrial rate, (B) tachycardia without changing the slope of diastolic depolarization, (C) increase in the slope of diastolic depolarization, not associated with tachycardia, (D) increase in both the slope and the rate, and (E) increase in the slope accompanied by arrhythmia. Electrical current sufficient to produce significant changes in the membrane potential and atrial rate in at least one cell was applied in the study for this classifi-

| Regions | I   | II  | III | IV  | V   | VI  |
|---------|-----|-----|-----|-----|-----|-----|
| No. of cells | 7   | 31  | 17  | 11  | 11  | 3   |
| A       | 4 (57) | 0 (0) | 3 (18) | 8 (73) | 3 (27) | 2 (67) |
| B       | 1 (14) | 4 (13) | 3 (18) | 2 (18) | 1 (9) | 0 (0) |
| C       | 0 (0) | 1 (3) | 1 (6) | 0 (0) | 4 (37) | 1 (33) |
| D       | 2 (29) | 12 (39) | 7 (41) | 1 (9) | 3 (27) | 0 (0) |
| E       | 0 (0) | 14 (45) | 3 (17) | 0 (0) | 0 (0) | 0 (0) |

a Regions of the S-A node as shown in the figure (VCS and VCI: superior and inferior vena cava).
b Types of the responses to intracellularly applied isoproterenol (see text).
c Number of cells which responded to injected isoproterenol (figures in parentheses represent percentage).
cation. The results are summarized in Table 1. As shown in the table, the S-A node was divided into 6 regions. Typical responses, (D) and (F), were mainly obtained from cells in region II, from which true pacemaker action potentials were most often recorded, as well as in the neighboring region III.

**Difference in the effects of intra- and extracellularly applied isoproterenol**

In Fig. 4 an increase in the atrial rate is plotted against the value of concentrations of isoproterenol applied intra- and extracellularly x current strength x duration of current application. Data presented here were obtained from region II. Values in the figure scattered considerably when isoproterenol was intracellularly applied (correlation coefficient; r = 0.146, P < 0.4), and showed a marked contrast to those obtained with extracellular application of the amine (r = 0.949, P < 0.01). Gradients of the regression lines for intra- and extracellular application of the amine were 1.75 and 22.6, respectively. The mean value of the latency for inducing tachycardia when isoproterenol was intracellularly applied

![Graph showing the positive chronotropic effect of intra- and extracellularly applied isoproterenol.](image-url)
was 60.2±8.2 sec (N=31) and that for extracellular application was 19.1±2.1 sec (N=10). The difference was statistically significant (P<0.005).

Four preparations were utilized for comparing the effects of intra- and extracellularly applied isoproterenol by the use of double barrel microelectrodes. In a typical experiment, extracellular application of isoproterenol close to nodal cells in region II by electrical current of 70 nA for 10 sec caused an increase in the atrial rate from 104 to 113 beats/min in association with increased slope of diastolic depolarization. When the same isoproterenol electrode was forwarded to impale the cell, the same current failed to cause changes in the membrane potential and atrial rate. Prolongation of the duration of current application to 30 sec caused increases in the slope of diastolic depolarization and the atrial rate from 104 to 110 beats/min. Similar results were obtained in the remaining 3 atria.

Effects of intracellularly applied noradrenaline

Intracellular application of noradrenaline failed to produce significant changes in the membrane potential and atrial rate in 12 S-A nodal cells from 4 preparations. However, in atria treated for 20 min with 10⁻⁶ M cocaine, the cells responded to intracellularly applied noradrenaline as did those to isoproterenol (Fig. 5). Latency for inducing tachycardia averaged 16.3±3.5 sec (N=9).

![Fig. 5](image)

DISCUSSION

The present study revealed that isoproterenol injected into S-A nodal cells caused a significant increase in the slope of diastolic depolarization, resulting in tachycardia. Conversion of latent pacemaker cells to a true pacemaker was also demonstrated. These results are consistent with those obtained when catecholamines are added to the bathing me-
dium (3, 4, 5) and sympathetic nerves innervating the S-A node are electrically stimulated (4). Since the effects of intracellularly applied isoproterenol were abolished by treatment with propranolol, an adrenergic mechanism would be involved in the changes in the membrane potential and atrial rate. The hypothesis that a major site of action of isoproterenol applied in this way locates on the outside of membranes of the nodal cells is supported by the following findings. (a) The intracellular application of the amine was appreciably less efficient in increasing the atrial rate than the extracellular application. Latency for inducing the tachycardia was markedly prolonged by injecting the amine intracellularly. Although the presence of monoamine oxidase has been demonstrated in cardiac muscle cells (8), isoproterenol is resistant to this enzyme (9). Thus, different effectiveness of intracellularly and extracellularly applied isoproterenol would not be associated with differences in degradation of the amine. Furthermore, the fact that isoproterenol is not actually taken up by adrenergic nerves (10) may favour the maintenance of a high amine concentration in the vicinity of receptor sites. (b) Membrane effects of intracellularly applied noradrenaline were produced only when the preparations were treated with cocaine which blocks the intraneuronal uptake of noradrenaline in the heart (11). This leads us to the assumption that the amine leaks out of the penetrated cell. (c) Intracellularly applied isoproterenol sometimes accelerated the atrial rate without increasing the slope of diastolic depolarization in the penetrated cell, shown as type B response in the Results. This phenomenon is considered to derive from a leakage of injected isoproterenol out of the penetrated cell, thus diffusing to and stimulating cells sensitive to the amine. Changes in the membrane potential during diastole in these cells may be adequate enough to produce tachycardia. However, electrotonic propagation of the potential changes may not be large enough to cause significant changes in the diastolic membrane potential in the penetrated cell. These findings, however, do not completely exclude a possible intracellular site of action of catecholamines.

Actions of isoproterenol when applied intracellularly may be limited to the penetrated cell and cells of a surrounding small area. Thus, this method of the amine application would be useful in investigating the regional difference in the responsiveness of S-A nodal cells to the amine. The present study demonstrated that the middle part of the S-A node, including the region II from which true pacemaker action potentials were usually recorded (termed "true pacemaker area") and the region III, was more sensitive to isoproterenol than that the upper (region IV) and the lower end (region I) of the S-A node. According to Lu and Brooks (5), the lower part of the cat's S-A node is more sensitive to noradrenaline and is controlled more efficiently by sympathetic nerves than the upper part including a true pacemaker area. They suggested that the shift of dominant pacemaker site from the upper part of the node to the lower part was partially responsible for the shortening of A-V conduction time during catecholamine administration or cardiac sympathetic nerve stimulation. Discrepancy between the former results and those herein may possibly be due to different species used (cat and rabbit) and different methods for determining the regional difference in sensitivity.
REFERENCES

1) Del Castillo, J., and Katz, B.: J. Physiol. 128, 157 (1955)
2) Matsuda, K.: Abstr. XXIII Intern. Physiol. Congr. Tokyo, 130 (1965)
3) West, T.C., Falk, G. and Corvoni, P.: J. Pharmacol. exp. Ther. 117, 245 (1956)
4) Toda, N., and Shimamoto, K.: J. Pharmacol. exp. Ther. 159, 298 (1968)
5) Lu, H.H., and Brooks, C. M.C.: Circulation, 40, 136 (1969)
6) Araki, T., and Otani, T.: J. Neurophysiol. 18, 472 (1955)
7) West, T.C.: J. Pharmacol. exp Ther. 115, 283 (1955)
8) Lowe, M.C., Reichenbach, D.D. and Horita, A.: Fedn Proc. 30, 223 (1971)
9) Graham, J.D.P.: Pharmacology for Medical Students, p. 33, Oxford University Press, London, New York and Toronto (1971)
10) Burgen, A.S.V. and Iversen, L.L.: Br. J. Pharmacol. Chemother. 25, 34 (1965)
11) Iversen, L.L.: The Uptake and Storage of Noradrenaline in Sympathetic Nerves, p. 151, Cambridge University Press, London and New York (1967)