Distribution and Metabolism of Norepinephrine, Cyclic AMP and Cyclic GMP in the Atrioventricular Conducting Tissue of the Bovine Heart

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SUMMARY

The distribution of norepinephrine (NE), cyclic AMP (cAMP) and cyclic GMP (cGMP) and the activities of related enzymes in the atrioventricular (A-V) conducting tissue of the bovine heart were examined. The concentration of NE in the atrium was about twice that in the ventricle. In the A-V conducting tissue, the concentration of NE was highest in the atrioventricular node (AVN) and lowest in the false tendon (FT), with intermediate levels in the bundle of His (HIS) and the right and left bundle branches (RLBB). The activity of monoamine oxidase (MAO) in the atrium was about 2.2 times that in the ventricle. In the A-V conducting tissue, the activity of MAO was highest in the HIS and lowest in the FT. The activity of catechol-o-methyltransferase (COMT) in the atrium and ventricle was similar, and that in the HIS was slightly, but not significantly, higher than that in other regions of the A-V conducting tissue. The concentration of cAMP in the ventricle was about twice that in the atrium. In the A-V conducting tissue, the concentration of cAMP was higher in the AVN and FT than in the HIS and RLBB. The distribution of adenylate cyclase (AC) was similar to that of NE. The phosphodiesterase (PDE) activity in the atrium and ventricle was similar. No significant difference was found in the level of PDE activity in different regions of the A-V conducting tissue. The concentration of cGMP was slightly, but not significantly, higher in the A-V conducting tissue than in the atrium or ventricle. In the A-V conducting tissue, the concentration of cGMP was highest in the FT and the concentrations in the HIS, RLBB and AVN were similar. These findings suggest that in the A-V conduction tissue, the regions that have the higher spontaneous pacemaker rates have higher NE content and AC activity, that is sensitivity to NE. Furthermore, the sensitivity for muscarinic cholinergic

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stimulation is higher in the conducting tissue (especially in the FT) than in the atrium and ventricle.

Additional Indexing Words:

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Catechol-o-methyltransferase Adenylate cyclase Phosphodiesterase
Atrioventricular conducting tissue

POSTGANGLIONIC adrenergic nerve fibers seem to reach all parts of the heart, but the densest innervation is found in the sinus node and AVN.¹⁾ The atria are also richly innervated by adrenergic fibers. In the ventricles, the adrenergic innervation is less dense and shows marked regional variation. The Purkinje system appears to be only sparsely innervated by sympathetic fibers.²⁾⁻⁴⁾ The sinus node, AVN and atria also receive an abundant supply of cholinergic fibers. Although sparse cholinergic innervation is present in the ventricles, numerous cholinergic nerve fibers are present in the Purkinje system.⁵⁾

These differences in the adrenergic and cholinergic innervation of the heart, with special regard to its conducting tissue, suggest that the distribution of norepinephrine (NE), cyclic AMP (cAMP) and cyclic GMP (cGMP) differs in various regions of the heart. Therefore, in the present study we examined the distribution of these substances and related enzymes in the atrioventricular (A-V) conducting tissue of the bovine heart.

MATERIALS AND METHODS

Atrial, ventricular and A-V conducting tissues were isolated from bovine hearts. The animals were stunned by a blow to the head and bled to death from the common carotid arteries in a slaughterhouse. After death, the hearts were promptly removed and brought to the laboratory on ice. The epicardial and endocardial tissues from the left atrium and left ventricle and the atrioventricular conducting tissue were excised as rapidly as possible (within 1 h after death) in a cold room. The A-V conducting tissue consisted of the atrioventricular node (AVN), the bundle of His (HIS), the right and left bundle branches (RLBB), and the false tendon (FT). S-Adenosyl-L-methionine-¹⁴C (58 mCi/mmol) was obtained from New England Nuclear Corporation, and L-epinephrine bitartrate from Sigma (St. Louis, MO, USA). All other compounds were obtained from Wako (Osaka, Japan) and were of the highest grade available.

Determination of NE:

Myocardial tissue (about 0.4 g) was homogenized in 10 vol of chilled

6% perchloric acid in a Polytron and the homogenate was centrifuged at 800 g for 20 min at 4°C. The catecholamine (CA) in the tissue extract was absorbed onto aluminium oxide and oxidized with potassium ferricyanide. The concentration of the resulting trihydroxyindole was measured by high-performance liquid chromatography⁶⁾ using a Shimadzu catecholamine analysis equipment system (Shimadzu, Kyoto, Japan).

MAO assay:

Myocardial tissue (0.5 g) was homogenized in 10 ml of chilled 0.25 M sucrose and centrifuged at 800 g for 10 min at 4°C. The supernatant was centrifuged at 12,000 g for 20 min at 4°C and the precipitate was suspended in 3 ml of 0.25 M sucrose. The suspension was used for assay of MAO. MAO activity was assayed with m-nitrobenzylamine as the substrate⁷⁾ with a MAO assay kit from Wako (Osaka, Japan). Enzyme activity is expressed in nmoles/mg protein/h.

COMT assay:

COMT activity was determined by the method of Axelrod.⁸⁾ Myocardial tissue (0.5 g) was homogenized in 1 ml of chilled 0.15 M KCl. A volume of 0.2 ml of homogenate was incubated with 0.1 ml of 0.5 M phosphate buffer, pH 7.9, 0.05 ml 2 M MgCl₂, 0.05 ml L-epinephrine bitartrate (0.5 μmole) and 0.01 ml S-adenosylmethionine-¹⁴C (1.0 mμmole) in a final volume of 0.5 ml. The incubation was carried out for 1 h at 37°C. The reaction was stopped with 0.5 ml of 0.5 M borate buffer, pH 10 and the normetanephrine-¹⁴C formed was extracted into 5 ml toluene isoamyl alcohol (3:2) by shaking for 15 min; volumes of 3 ml of the organic phase were transferred to counting vials and radioactivity was determined in a liquid scintillation spectrometer. Enzyme activity is expressed in pmoles of metanephrine formed/mg protein/h.

Determinations of cAMP and cGMP:

Myocardial tissue (about 0.1 g) was homogenized in 10 vol of chilled 6% perchloric acid and the homogenate was centrifuged at 1,000 g for 10 min at 4°C. The supernatant was neutralized with 60% KOH and centrifuged at 1,000 g for 10 min at 4°C, and the resultant supernatant was used for determinations of cAMP and cGMP. cAMP and cGMP were measured by radioimmunoassay of succinylated cAMP and cGMP as reported by Honma et al⁹⁾ using Yamasa cAMP and cGMP kits, respectively.

AC assay:

AC activity was determined by a modification of the method of Drummond and Duncan.¹⁰⁾ Myocardial tissue was homogenized in 10 vol of chilled 10 mM Tris-HCl buffer, pH 7.5, in a Polytron. The homogenate was strained through a coarse nylon sieve and then centrifuged at 1,000 g for 15 min at 4°C. The precipitate was washed twice with the original volume of

buffer with centrifugation. The resulting pellet was suspended in 5 vol of buffer (based on the original tissue weight) in a glass homogenizer with a loosely fitting Teflon pestle. Preparations were then kept on ice and used within 15 min.

The reaction mixture (in a total volume of 0.4 ml) consisted of 30 mM Tris-HCl, pH 7.5, 5 mM MgCl_2 , 10 mM theophylline, 1 mM EDTA, 10 mM phosphoenolpyruvate, 0.05 mg/ml pyruvate kinase, 2.5 mM ATP and enzyme (usually 100 to 400 μg of protein). All solutions were previously adjusted to pH 7.5. After addition of all components except enzyme, the assay tubes were equilibrated at 37°C for 2 min. The reaction was started by adding suitably diluted enzyme (0.05 ml) and incubations were carried out for 10 min at 37°C. The reaction was stopped by placing the tubes in a boiling water bath for 3 min. Denatured protein was removed by centrifugation at 9,000 g for 5 min, and the cAMP content of the supernatant was determined by the method of Honma et al.⁹⁾ Enzyme activity is expressed in pmoles of cAMP produced/mg protein/min.

PDE assay:

The enzyme solution was prepared by the same method as for preparation of AC. PDE activity was determined by the method of Butcher and Sutherland¹¹⁾ as follows: The reaction mixture contained 9.6 mM cAMP, 24 mM MgSO_4 and 0.16 M Tris-HCl buffer, pH 7.5, with a suitable dilution of the test PDE sample in a total volume of 0.8 ml. This mixture was incubated at 37°C for 10 min, and then the reaction was stopped by heating the vessels at 100°C for 2 min. The vessels were cooled and then incubated with 0.1 ml of *Crotalus atrox* venom (1 mg of venom in 20 ml of 0.1 M Tris-HCl buffer, pH 7.5) at 30°C for 10 min. The reaction was stopped by adding 0.1 ml of chilled 55% trichloroacetic acid. The resulting precipitate was removed by centrifugation, and inorganic phosphate in aliquots of the supernatant were measured by the method of Fiske and Subbarow.¹²⁾ Enzyme activity is expressed in μmoles of 5'-AMP liberated/mg protein/min.

Protein concentration was measured by the method of Lowry et al.¹³⁾

Analysis of data:

Data are given as means $\pm$ standard errors of means. Statistical comparisons were made by Student's t-test. The confidence level of significance for all statistical analyses was $p < 0.05$.

RESULTS

1. NE concentrations

Figure 1 shows the NE concentrations of various myocardial tissues.

Fig. 1. Concentrations of norepinephrine in atrial, ventricular and atrio-ventricular conducting tissues. Columns and bars represent means and one standard error of means. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. NS = not significant; n = number of experiments; Atr = atrium; Vent = ventricle; AVN = atrioventricular node; HIS = bundle of His; RLBB = right and left bundle branches; FT = false tendon; EN = endocardium; EP = epicardium.

The NE concentration of the atrial tissue was about twice that of the ventricular tissue. In the atrial tissue, the concentration in the epicardial tissue ($1.80 \pm 0.07 \mu\text{g/g}$ wet tissue) was higher than that in the endocardial tissue (1.54 ± 0.04), but in the ventricular tissue there was no difference in the concentrations in the epicardial tissue (0.71 ± 0.04) and the endocardial tissue (0.81 ± 0.06). In the A-V conducting tissue, the NE content was highest in the AVN (1.07 ± 0.07) and lowest in the FT (0.21 ± 0.01), with intermediate levels in the HIS (0.81 ± 0.05) and the RLBB (0.70 ± 0.11).

2. MAO and COMT activities

The MAO activity in the atrial tissue (212.8 ± 24.6 nmoles/mg protein/h) was about 2.2 times that of the ventricular tissue (95.3 ± 10.6), as shown in Fig. 2. In the A-V conducting tissue, MAO activity was highest in the HIS (89.8 ± 3.9) and lowest in the FT (61.6 ± 2.8), with intermediate activities in the AVN (69.5 ± 5.0) and the RLBB (75.9 ± 3.9).

COMT activity of the atrial tissue (211.3 ± 26.2) was slightly, but not significantly, higher than that of the ventricular tissue (171.7 ± 23.1), as shown in Fig. 3. In the A-V conducting tissue, the COMT activity in the HIS (325.5 ± 87.9) was slightly, but not significantly, higher than those in the other parts.

3. cAMP concentrations

Figure 4 shows the cAMP concentrations of various myocardial tissues. The cAMP concentration of the ventricular tissue was about twice that of the

Fig. 2. Monoamine oxidase activities in atrial, ventricular and atrio-ventricular conducting tissues. Columns and bars represent means and one standard error of means. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. NS=not significant; n=number of experiments. Other abbreviations are as for Fig. 1.

Fig. 3. Catechol-o-methyltransferase activities in atrial, ventricular and atrioventricular conducting tissues. Columns and bars represent means and one standard error of means. * $p < 0.05$. NS=not significant; n=number of experiments. Other abbreviations are as for Fig. 1.

atrial tissue. There were no differences between the concentrations in the endocardial tissue (1.89 ± 0.14 nmoles/g wet tissue) and the epicardial tissue (1.77 ± 0.12) of the atrium or between those in the endocardial tissue (3.45 ± 0.54) and the epicardial tissue (3.21 ± 0.25) of the ventricle. In the A-V conducting tissue, the cAMP concentrations in the AVN (0.59 ± 0.05) and the FT (0.71 ± 0.10) were higher than those in the HIS (0.43 ± 0.03) and RLBB (0.46 ± 0.04).

Fig. 4. Concentrations of cyclic AMP in atrial, ventricular and atrio-ventricular conducting tissues. Columns and bars represent means and one standard error of means. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. NS = not significant; n = number of experiments. Other abbreviations are as for Fig. 1.

4. AC and PDE activities

The AC activities in the endocardial tissue (19.44 ± 2.24 pmoles cAMP/mg protein/min), and the epicardial tissue (17.28 ± 0.84) of the atrium were about 3 times those in the endocardial tissue (6.07 ± 1.17) and the epicardial tissue (6.78 ± 1.31) of the ventricle, as shown in Fig. 5. In the A-V conducting tissue, the AC activity was highest in the AVN (13.26 ± 2.39), lowest in the FT (1.33 ± 0.28) and intermediate in the HIS (5.20 ± 0.59) and the RLBB (4.97 ± 1.85).

The PDE activities in the atrial tissue (endocardial tissue, 11.91 ± 2.35 μ moles AMP/mg protein/min; epicardial tissue, 9.87 ± 1.75) and the ventricular tissue (endocardial tissue, 12.00 ± 1.56 ; epicardial tissue, 11.85 ± 1.12) were similar, as shown in Fig. 6. The PDE activities in each portion of the A-V conducting tissue were significantly lower than those of the atrial and ventricular tissues. In the A-V conducting tissue, the PDE activity of the AVN (1.35 ± 0.44) was slightly, but not significantly, lower than those of the HIS (2.22 ± 0.69), RLBB (4.13 ± 0.90) and FT (1.91 ± 0.29).

5. cGMP concentrations

Figure 7 shows the cGMP concentrations of various myocardial tissues. In the atrial tissue, the concentration of the epicardial tissue (10.77 ± 1.41 pmoles/g wet tissue) was higher than that of the endocardial tissue (5.54 ± 0.30). In the ventricular tissue there was no difference in the concentrations in the endocardial tissue (7.23 ± 0.24) and epicardial tissue (8.21 ± 1.19). In the A-V conducting tissue, the cGMP concentration was highest in the FT (24.90 ± 2.27). The concentrations in the HIS (18.58 ± 4.44), RLBB ($10.03 \pm$

Fig. 5. Adenylate cyclase activities in atrial, ventricular and atrioventricular conducting tissues. Columns and bars represent means and one standard error of means. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. NS = not significant; n = number of experiments. Other abbreviations are as for Fig. 1.

Fig. 6. Phosphodiesterase activities in atrial, ventricular and atrioventricular conducting tissues. Columns and bars represent means and one standard error of means. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. NS = not significant; n = number of experiments. Other abbreviations are as for Fig. 1.

1.35) and AVN (8.98 ± 1.44) were not significantly different.

DISCUSSION

Catecholamines are widely distributed in animals, especially in nerve cells. NE is mainly localized in peripheral postganglionic sympathetic nerves.

Fig. 7. Concentrations of cyclic GMP in atrial, ventricular and atrio-ventricular conducting tissues. Columns and bars represent means and one standard error of means. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. NS = not significant; n = number of experiments. Other abbreviations are as for Fig. 1.

The NE concentration in some sympathetic ganglia is as high as $100 \mu\text{g/g}$, while in sympathetically innervated tissues its concentration ranges from 0.1 to $2 \mu\text{g/g}$.^{14),15)}

In the present study, the NE concentrations in various regions of the heart were found to range from 0.21 to $1.80 \mu\text{g/g}$ wet tissue. In the heart, the NE concentration was highest in the atrium (especially the epicardial tissue). In the A-V conducting tissue, the NE concentration was highest in the AVN and lowest in the FT, with intermediate concentrations in the HIS and RLBB. The NE distribution seemed to be almost consistent with the density of postganglionic adrenergic nerve fibers in the heart.¹⁾ The NE concentration in the AVN was lower than that in the atrium, but because the conducting tissue of bovine heart contains more connective tissue (about 60%) than ordinary myocardial tissue (less than 10%),¹⁶⁾ the actual NE concentrations in the AVN and other regions of the A-V conducting tissue are probably higher than the observed values.

The activities of MAO and COMT were higher in the atrium than in the ventricle. In the A-V conducting tissue, the activity of MAO was in the order $\text{HIS} > \text{AVN}$ and $\text{RLBB} > \text{FT}$. The activity of COMT in the HIS tended to be high in the A-V conducting tissue. These results suggest that the metabolism of NE is more rapid in the atrium than in the ventricle and

that in the A-V conducting tissue it is most rapid in the HIS.

NE affects the heart by interaction with β -receptors. Stimulation of β adrenergic receptors results in activation of AC via a regulatory coupling protein that binds guanine nucleotides.^{17),18)} The activation of AC leads to an increase in intracellular cAMP, which mediates many of the physiologic effects of stimulation of β adrenergic receptors by NE.^{19),20)} Probably by action on cAMP-dependent protein kinases, cAMP triggers phosphorylation reactions of proteins that directly or indirectly increase the transmembranal influx of Ca^{2+} ; cAMP may affect fluxes of other ions as well, accounting for changes in the electrophysiological behavior of myocardial cells.¹⁾

Previously²¹⁾ we reported that the concentration of cAMP and the activity of AC were higher in the AVN than in the HIS and RLBB. In the present study, we examined the distribution of cAMP, AC and PDE in the atrium, ventricle and A-V conducting tissue consisting of the AVN, HIS, RLBB and FT. The activity of AC was higher in the atrium than in the ventricle, and in the A-V conducting tissue its activity was in the order AVN > HIS and RLBB > FT. These findings show that the distribution of AC activity was consistent with that of NE. Therefore, in the A-V conducting tissue, the regions that have the higher spontaneous pacemaker rates have higher NE contents and AC activity, that is sensitivity to NE.

The cAMP concentration was found to be lower in the atrium than in the ventricle. In the A-V conducting tissue, the cAMP concentrations in the AVN and FT were higher than those in the HIS and RLBB. There was no significant difference in the PDE activity in the atrium and ventricle. The PDE activity was similar in the various regions of the A-V conducting tissue. Thus, disparity was found between the AC activity and the cAMP concentration. Further studies of additional factors regulating the AC activity and ATP level are necessary to explain this disparity.

Many studies in a variety of tissues have shown that activation of muscarinic receptors leads to an increase in the tissue concentration of cGMP.²²⁾ Thus under appropriate conditions, possibly related to the concentration of Ca^{2+} in the extracellular medium, activation of muscarinic receptors certainly increases the myocardial tissue cGMP level. The present study showed that the cGMP concentration in the A-V conducting tissue tended to be higher than those in the atrium and ventricle. In the A-V conducting tissue, the cGMP concentration was highest in the FT. These results suggest that the sensitivity to muscarinic cholinergic stimulation is higher in the conducting tissue than in the atrium and ventricle and that in the A-V conducting tissue it is highest in the FT. However, the physiological significance of this elevation in the cGMP level is controversial.²³⁾

The present study demonstrated the heterogeneous distributions of NE, cAMP, cGMP and the activities of related enzymes in the A-V conducting tissue. Studies of the distribution and physiological properties of adrenergic and muscarinic receptors are in progress.

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