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# ACUTE EFFECTS OF NICOTINE ON GLYCOLYSIS OF RAT MYOCARDIUM WITH SPECIAL REFERENCE TO CARDIOVASCULAR EFFECTS

(glycolysis intermediates/myocardium/nicotine/rat)

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Acute effects of nicotine bitartrate (Nic.) on glycolysis intermediates concentrations in the heart of female rat were studied with special reference to cardiovascular effects. Biphasic (fast negative and slow positive) inotropic and chronotropic effects of Nic. (100 μM) on spontaneous beating atrial preparations were observed. The negative inotropic and chronotropic effects were inhibited by atropine (15nM) and positive inotropic effect was inhibited by propranolol (150nM) significantly, suggesting that fast negative effects were mediated through muscarinic cholinergic receptors and slow positive inotropic effect was through β-adrenergic receptors. Positive chronotropic effect was not inhibited significantly by 150 nM propranolol. At the plateau phases of positive response by nicotine with or without atropine, glycolysis intermediates in the atrial preparation was measured and no significant change was observed. Blood pressure decreased by 5-min-infusion of Nic. (20 μg) under pentobarbital anesthesia, which disagreed with the reports using dog and cat. At 5 and 20 min after the start of Nic. infusion, no significant change on glycolysis intermediates concentrations was observed in myocardium. These results suggested that the effects of nicotine on glycolysis in myocardium did not appear significantly by acute treatment.

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Smoking is considered as an important risk factor of myocardiac convulsion (1-3). However, details of the mechanism of the effect of smoking on ischemic heart failures, such as myocardiac convulsion, were unknown. It is well known that intravenous administration of nicotine, which is one of the main

substance of smoke, induced hypertension in dog and cat (4, 5). Cardiovascular effects of nicotine and the reduction of oxygen supply with an enhancement of monooxycarbonate coupling with hemoglobin by smoking have been considered to relate to ischemic heart failure (2).

An increase of glycolysis intermediates and lactate in myocardium during acute experimental ischemia was observed and it was suggested that such enhancement resulted cardiac acidosis and hypofunction of heart would be followed (6, 7). Then, glycolysis pathway is considered to have an important role on the development of myocardiac ischemia. However, reports on the effects of nicotine or smoking on myocardiac metabolism are fragmentary. We found a significant reduction of glycolysis intermediates concentrations in rat myocardium after 2 weeks administration of nicotine with water (8). The question arisen was whether such an effect was also observed by acute treatment.

In the present study, cardiovascular effects of acute nicotine administration on rats were studied, because reports were fragmentary in this species. Then, effects of nicotine on glycolysis intermediates concentrations at the time when cardiovascular effects showed the maximum were studied.

## MATERIALS AND METHODS

### Inotropic and chronotropic effects of nicotine on isolated rat atrial preparation

Sprague-Dawley (SD) female rats (9-11 weeks of age; ca. 200 g) were obtained from a commercial source. After decapitation, atria were isolated and both left and right auricles were fixed with threads. The hole atrial preparation was fixed vertically under a resting tension of 1 g in a 20 ml tissue bath. Krebs-Henseleit solution was aerated with 95% $O_2$-5% $CO_2$ and kept at 30 °C. Krebs solution was changed every 5 min and the experiment was started after 25 min. Inotropic effects and chronotropic effects of nicotine bitartrate (1 or 100 μM) were measured isometrically for 5 min by polygraph system (Biophysiograph, 180 system, NE-Sanei) with a transducer (TMI, NE-Sanei). Immediately after the measurement, the tissue was frozen rapidly with aceton and Dry Ice. Atria from 2 rats were pooled for a sample for metabolites assay.

Atropine (15 nM) and/or propranolol (150 nM) was added to the bath just after tension loading. For the control, saline was added.

Acute effects of nicotine on rat blood pressure

The same strain female rats were anesthetized with pentobarbital (4 mg/ 100 g body weight, i.p.) and polyethelene tube (PE 10) was cannulated to common carotid artery. The blood pressure and the heart rate were measured with polygraph system (RM-25, Nihon Koden) with a pressure transducer (MPU-0.5, Nihon Koden). From a catheter in femoral vein, 20 μg of nicotine bitartrate in 500 μl of Krebs solution was infused for 5 min and 100 μl/min of Krebs solution was infused before and after the nicotine administration. As the control, 500 μl of Krebs solution was applied in the same way. At 5 or 20 min after the start of nicotine infusion, the whole heart was isolated and frozen rapidly. For the control, the preparation at 5 min after the start of the infusion of Krebs solution was used.

Measurement of glycolysis intermediates concentrations in myocardium

After weighing the frozen tissue, 2 fold of 30% perchloric acid was added, homogenized with an ultra disperser (LK21, Yamato Co.) and it centrifuged at 3,000 rpm for 15 min (deproteinization procedure). The superfusate was neutralized with potassium hydroxide and centrifuged again. The superfusate was used as the intermediates sample. The sample was stored at -80 °C until the measurement. Each intermediate concentration was measured by an enzyme method (9, 10). The precipitate of deproteinization procedure was used for protein determination by Lowry's method (11).

Reagents

Reagents and enzymes were purchased from following commercial sources; NAD, NADP and NADH from Kojin Co., Ltd. (Tokyo), glucose-6-phosphate dehydrogenase (from yeast), glycerol-3-phosphate dehydrogenase (from rabbit muscle), lactate dehydrogenase (from pig heart), pyruvate kinase (from pig heart) and enolase (from yeast) from Oriental Yeast Co., Ltd. (Tokyo), phosphoglucose isomerase (from bakers yeast), phosphoglucomutase (from yeast), hexokinase (from yeast), triosephosphate isomerase (from rabbit

muscle), aldolase (from rabbit muscle) and phosphoglyceromutase (from rabbit muscle) from Sigma chemical Co. (St. Lewis).

Statistical analysis

For the comparison between the control and nicotine administered group or nicotine-administered group and blocker(s)-treated nicotine-administered group, Student's t-test was applied.

## RESULTS

Effects of nicotine on isolated rat atrial preparation

The stroke just before nicotine administration was 228 ± 17 mg (n=15) and the beating rate was 178 ± 7 beat/ min (n=15). Administration of propranolol did not affect on initial beating (Table 1). Administration of atropine reduced beating force significantly, but did not affect on beating rate. Beating force of the control group tended to elevate and the beating rate tended to reduce during the 5 min measurement (Fig. 1a)

After 100 μM nicotine administration, the stroke began to decrease immediately and it reduced to about 80% of the initial levels at 30 second after the administration. Then it began to recover, became larger than the initial level at 1.5 min and reach to the maximum (ca. 180%) at 3 to 5 min (Fig. 1b). The fast negative inotropic effects were significantly inhibited by atropine, however, slow positive inotropic effects were not enhanced (Fig. 1c). The slow positive inotropic effects were inhibited by propranolol significantly, however, the fast negative effect was not enhanced (Fig. 1d). The stroke change after nicotine administration with both atropine and propranolol treatment seemed to be the same as the control (Fig. 1e).

After 100 μM nicotine administration, the beating rate also decreased to about 90% of the initial levels at 30 second, then it became larger than the initial level at 2.5 min and reach to the maximum (ca. 110%) at 3 to 5 min (Fig. 1b). The fast negative chronotropic effects were significantly inhibited by atropine as well as the inotropic effect (Fig. 1c), however, the slow positive inotropic effects were not inhibited by propranolol significantly (Figs. 1d, 1e). The slow positive chronotropic effects of nicotine tended to be enhanced by atropine, however, it was not significant (Fig. 1d). After 1 μM nicotine administration, no

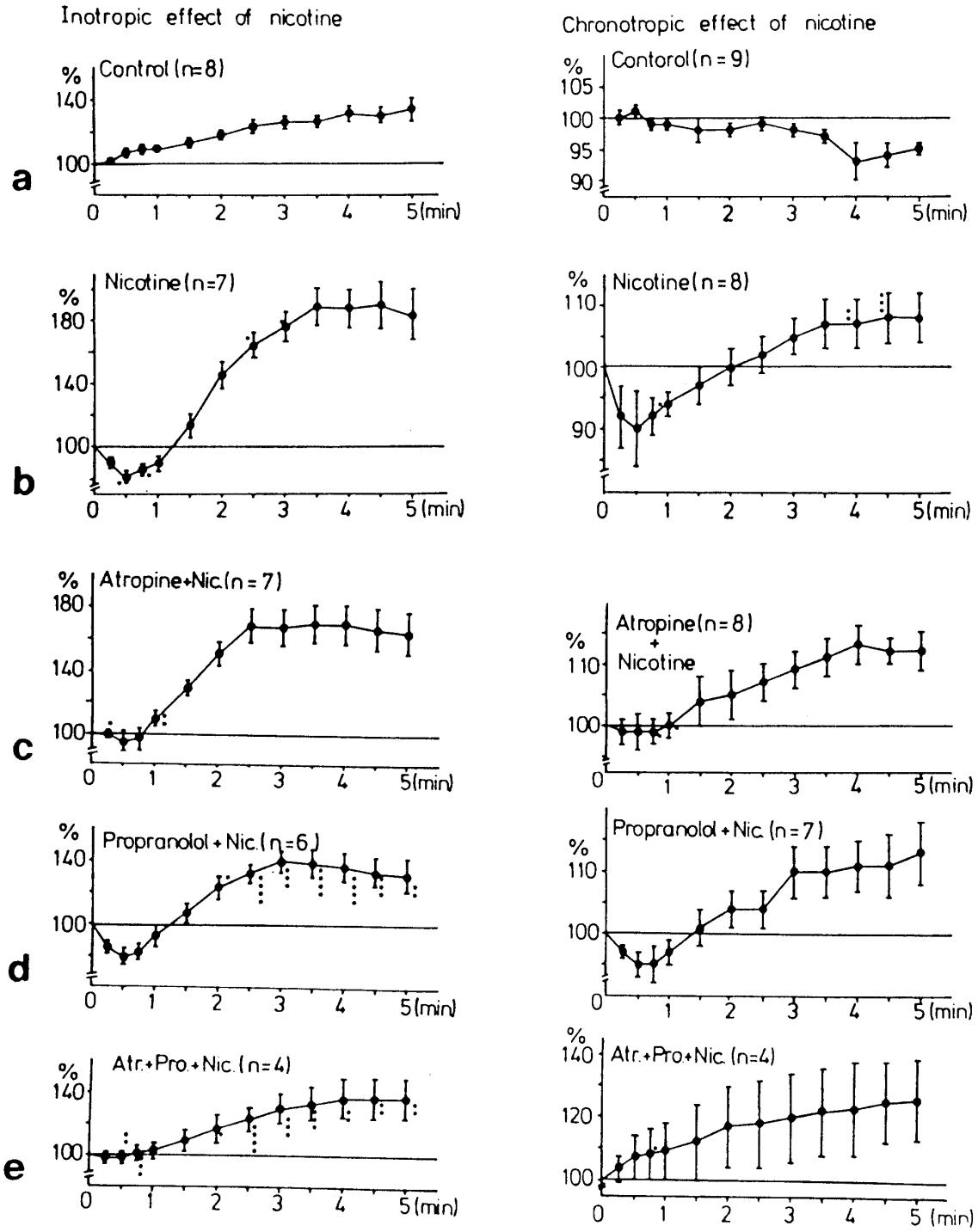
Inotropic effect of nicotine
Chronotropic effect of nicotine
a
Control (n=8)
Contorol (n = 9)
b
Nicotine (n=7)
Nicotine (n = 8)
c
Atropine+Nic. (n = 7)
Atropine (n=8) + Nicotine
d
Propranolol + Nic. (n=6)
Propranolol + Nic. (n = 7)
e
Atr.+Pro.+Nic. (n=4)
Atr.+Pro+Nic. (n=4)
%
0 1 2 3 4 5 (min)

Figure 1. Effects of nicotine bitartrate on rat atrial preparation in vitro. The stroke and beating rate just before nicotine administration was taken as 100 % and relative stroke (left) and beating rate (right) were shown. a; Control group, b; Nicotine (100 μM) treated group, c; Nicotine (100 μM) treated group with atropine (15 nM) pretreatment, d; Nicotine (100 μM) treated group with propranolol (150 nM) pretreatment, e; Nicotine (100 μM) treated group with atropine (15 nM) and propranolol (150 nM) pretreatment.
Vertical bars indicate standard error.
Statistical analysis has been done between control group (a) vs nicotine treated group (b) or nicotine treated group (b) vs nicotine treated groups with atropine and/or propranolol pretreatment (c-e) of the same time course.
* $p < 0.05$, ** $p < 0.025$, *** $p < 0.01$, **** $p < 0.005$.

significant inotropic or chronotropic effect was detected.

Concentrations of glucose, fructose-1,6-diphosphate (FDP), pyruvate and lactate in atrial preparation of the control, nicotine treated and nicotine and atropine treated groups were shown in Table 2. No significant difference was observed in intermediates concentrations among 3 groups.

Table 1. The stroke and beating rate just before nicotine administration.

| Group | Number of Experiment | Stroke (mg) | Beating rate (/min) |
|---|---|---|---|
| a | 8 | 235 ± 18 | 175 ± 11 |
| b | 7 | 220 ± 31 | 181 ± 8 |
| c | 7 | 150 ± 21 * | 183 ± 9 |
| d | 6 | 208 ± 53 | 175 ± 11 |
| e | 4 | 194 ± 40 | 169 ± 13 |

a; Control group, b; Nicotine (100 μM) treated group, c; Nicotine (100 μM) treated group with atropine (15 nM) pretreatment d; Nicotine (100 μM) treated group with propranolol (150 nM) pretreatment, e; Nicotine (100 μM) treated group with atropine (15 nM) and propranolol (150 nM) pretreatment.
mean ± standard error.
* $p < 0.05$ compared with the control.

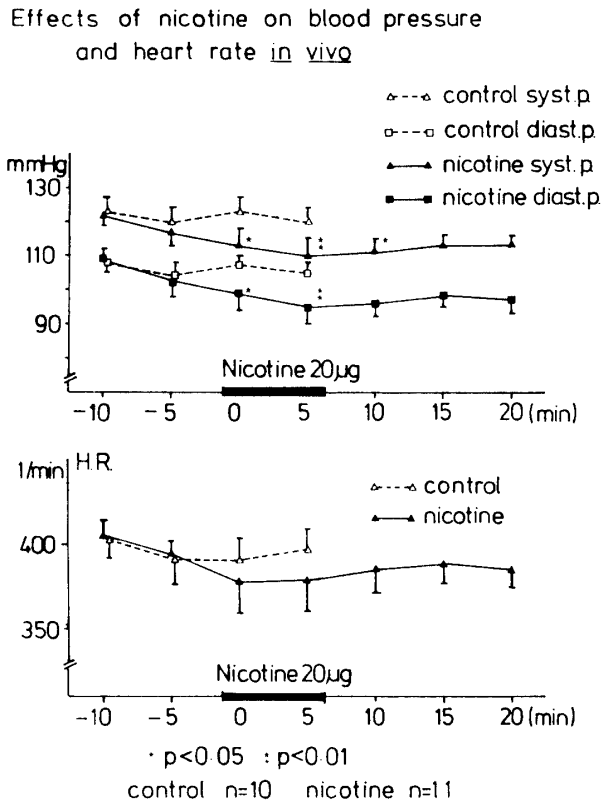


Figure 2. Effects of nicotine bitartrate infusion (thick bar) on systolic (syst.) and diastolic (diast.) blood pressure (upper) and heart rate (lower) of rats. Animal numbers were 10 for control and 11 for nicotine treatment. Vertical bars indicate standard error. * $p < 0.05$, ** $p < 0.01$

Table 2. Effects of nicotine on glycolysis in rat atrium in vitro.

| | Control n=4 | Nicotine n=4 | Atropine + Nicotine n=4 |
|---|---|---|---|
| glucose* | 26.6 ± 3.6 | 28.2 ± 3.9 | 25.6 ± 1.5 |
| fructose-1,6-diP** | 334 ± 94 | 400 ± 99 | 372 ± 102 |
| pyruvate** | 677 ± 153 | 774 ± 95 | 812 ± 167 |
| lactate* | 26.1 ± 4.2 | 26.1 ± 4.9 | 27.3 ± 5.8 |

* μmol/g protein ** nmol/g protein
mean ± standard error.
P means phosphate

Table 3. Effects of nicotine on glycolysis in rat heart in vivo.

| | Control (n=5) | Nicotine 5 min (n=6) | Nicotine 20 min (n=5) |
|---|---|---|---|
| glucose* | 14.8 ± 1.5 | 14.3 ± 1.0 | 12.1 ± 1.2 |
| glucose-1-P** | 358 ± 121 | 572 ± 200 | 271 ± 183 |
| glucose-6-P** | 2672 ± 913 | 3088 ± 686 | 2299 ± 532 |
| fructose-6-P** | 836 ± 298 | 1203 ± 390 | 929 ± 256 |
| fructose-1,6-diP** | 1331 ± 201 | 1149 ± 136 | 886 ± 147 |
| dihydroxyacetone-P** | 751 ± 120 | 552 ± 141 | 588 ± 82 |
| 3-phosphoglycerate** | 957 ± 79 | 930 ± 111 | 874 ± 112 |
| pyruvate** | 1121 ± 109 | 978 ± 197 | 835 ± 147 |
| lactate* | 81.8 ± 6.1 | 91.1 ±11.7 | 78.6 ± 7.2 |

* μmol/g protein ** nmol/g protein
mean ± standard error.
P means phosphate.

## Effects of nicotine infusion

Both systolic and diastolic blood pressure began to decrease after nicotine infusion and it reached to the maximum at 5 min. The systolic blood pressure decreased 6.6 ± 2.3 mmHg compared with the initial values and the diastolic pressure decreased 7.6 ± 2.4 mmHg (Fig. 2). After the cessation of nicotine infusion, the pressure began to recover slowly. The heart rate tended to decrease, however, it was not significant.

No significant difference was observed in intermediates concentrations of each groups (Table 3), however, concentrations tended to decrease in nicotine treated group at 20 min after the administration. Concentration of lactate in every group was over 80 % of total glycolysis intermediates content. Intermediate concentrations from glucose to fructose-6-phosphate (F6P) in nicotine treated group at 5 min after the start of infusion tended to be higher and those below FDP in the pathway tended to be smaller compared with the control values, however, they were not significant. Ratio of FDP to F6P, which was considered as a parameter for phosphofructokinase (12), for nicotine treated group at 5 min after the start of infusion was 1.50 ± 0.65 and that for the control group was 1.92 ± 0.37.

## DISCUSSION

Biphasic (fast negative and slow positive) effects of nicotine were shown in atrial preparation. The negative effects was inhibited by atropine, suggesting that it was mediated by muscarinic cholinergic receptors. The positive one was inhibited by propranolol suggesting that it was mediated by β adrenergic receptors. Biphasic chronotropic effects of nicotine were also shown and it was suggested that the fast negative effects were mediated by muscarinic cholinergic receptors. Positive chronotropic effect was not inhibited significantly by 150 nM propranolol. The present results agreed with the previous papers using various species (13, for review, see 14).

Blood pressure decreased and heart rate tended to decrease by nicotine infusion in the present study. Decreases of blood pressure and heart rate by nicotine infusion from external carotid artery of rabbit under pentobarbital anesthesia were reported (15). On the contrary, increase of blood pressure and no change in heart rate by nicotine injection from veins were reported in dog (4, 16) and cat (5). A phasic enhancement of blood pressure by nicotine injection from femoral vein in pentobarbital anesthetized rats was also reported by von Borstel et al. (17). Cardiovascular effects of nicotine should be different by species and the method of administration.

In the present study, decrease of the blood pressure by nicotine was observed in rats, however in vitro results showed long term positive inotropic and chronotropic effects. Hypotensive effects of nicotine in rabbit was reported to be mediated by the stimulation of chemoreceptors in carotid artery by nicotine (15). The present hypotensive effects in rats may be mediated by the same mechanism. Further studies are necessary to clarify the mechanism.

Preparations for the measurement of glycolysis intermediates concentration after in vitro study were considered to be those fully stimulated by nicotine (nicotine group) and those stimulated by nicotine without negative effects by atropine treatment (atropine+nicotine group). On the other hand, preparations for intermediates concentration measurement after blood pressure measurement was those at 5 min when the response showed the

maximum and those at 20 min when the response began to recover.

The glycolysis intermediates concentrations in in vitro atrial preparation and in vivo whole heart preparation were much different especially in glucose and lactate concentrations. In a separate study, atrial and ventricular glycolysis intermediates concentrations of Wistar strain rats were measured separately without nicotine treatment and the difference on concentrations were less than 2 times and lactate concentrations were over 80% in both preparations (unpublished observations). The present results for whole heart preparation were well agreed with the concentrations of ventricular preparations, and in some way atrial preparations, of Wistar rats. Incubation of aerated Krebs' solution containing glucose affect on glycolysis intermediates concentrations of the present in vitro preparation.

Rats glycolysis intermediates concentrations were much different by the methods for preparations. The ratio of FDP/F6P and lactate/pyruvate were 1.92 $\pm$ 0.37 and 75.7 $\pm$ 9.1 in the present whole heart preparations which prepared after blood pressure measurement under anesthesia and frozen with aceton-Dry Ice. Kraupp *et* al. (12) measured glycolysis intermediates concentrations in some experimental conditions and the values in the present experiment were not similar to the values 0.12 and 9.5 in the rats heart preparation under artificial respiration with anesthesia but near to the values 1.2 and 119 in the rats heart preparation under hypoxia condition.

In the present study, no significant changes on glycolysis intermediates concentration of rat myocardium was detected, although inotropic and chronotropic effects in atrial preparations and hypotensive effects *in vivo* by nicotine was apparent. Thus, it was suggested that acute application of nicotine did not affect on glucolysis intermediates concentration in myocardium. The intermediates concentrations above and below phosphofructokinase at 5 min after the start of nicotine infusion tended to be different compared with saline infusion group. Furthermore, the tendency of reduction of intermediates at 20 min after the start of nicotine infusion was observed. Recently we found by $^{31}P$ magnetic resonance spectrum measurement that creatinine phosphate content in myocardium reduced significantly after nicotine infusion (18). These suggest the possibility that longer time administration of nicotine may show a significant effect on glycolysis pathway in myocardium. In fact, administration of

nicotine for 2 weeks with water induced significant reduction of glycolysis intermediates concentration in myocardium (8).

In conclusion, although cardiovascular effects were observed by acute infusion of nicotine, glycolysis intermediates concentrations were not significantly affected.

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