Effects of Formaldehyde on Cardiac Function

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Abstract—This investigation examined the effect of formaldehyde (HCHO) on cardiac function in *in vitro* cardiac preparations and *in situ* hearts of guinea pigs and rabbits. Though HCHO (0.2–4 mg/kg, i.v.) produced noticeable bradycardiac and negative inotropic responses in anesthetized guinea pigs and rabbits, the responses to HCHO were far less in isolated guinea pig auricles and perfused hearts (Langendorff's preparations). The inhibitory responses to HCHO in the isolated auricles and perfused hearts were obtained at concentrations 200–400 times and 4–8 times higher than the blood concentrations attained in anesthetized animals, respectively. The responses in the isolated preparations were not significantly affected by propranolol. The bradycardiac response to the intravenously administered HCHO in anesthetized animals was not significantly affected by atropine or vagotomy, but was markedly attenuated by propranolol, reserpine or surgical denervation of the heart. These results indicate that the direct action of HCHO on the heart plays only a small role in the negative chronotropic response to HCHO in anesthetized animals. Furthermore, the negative chronotropic effect of HCHO in animals seems to be caused mainly by the inhibition of sympathetic nervous activity through the central nervous system.

Formaldehyde (HCHO) is a highly reactive chemical and has various effects on living organisms such as sterilization, irritation of the eyes, skin and respiratory tract, and mutagenic and carcinogenic actions (1–20). Furthermore, intravenously administered HCHO has been reported to produce transient reductions in heart rate, blood pressure and respiratory depression in anesthetized animals such as guinea pigs, rabbits and dogs (21–24). We have previously reported that the hypotension induced by HCHO results from a combination of direct relaxation of vascular smooth muscles and inhibition of the sympathetic nervous system, and the direct vasodilator action plays a larger role in the hypotension than the sympathetic inhibition (22, 25). There are several reports describing the effects of HCHO on cardiac function, but the mechanism of the decrease in heart rate induced by intravenously administered HCHO in anesthetized animals have not been thoroughly studied so far (26–29). In this investigation, we studied the mechanism of the HCHO

effect on cardiac function.

Materials and Methods

Animals: Male Japanese albino rabbits weighing 2.4–3.4 kg and Hartley strain guinea pigs weighing 400–600 g (Shizuoka Laboratory Animal Center) were used for the present study. The animals were maintained in a room at $22 \pm 2^\circ\text{C}$ and were given laboratory chow (CR-1 or CG-3, Clea Japan Inc.) and tap water *ad libitum*.

Measurement of heart rate and blood pressure in anesthetized animals: Twenty-six rabbits and thirty-two guinea pigs were anesthetized with urethane (1.0–1.2 g/kg, i.p.), and the following parameters were recorded simultaneously with a multipurpose polygraph (Nihon Kohden, RM-6000): 1) blood pressure (BP) through an arterial catheter inserted into the left femoral artery, 2) heart rate (HR) through a cardi tachometer (Nihon Kohden, AT-600G) triggered by ECG, and 3) respiratory movement (RM) of the thorax wall by means of a thoracic pickup (Nihon Kohden,

TR-601T). Drugs were given through a cannula inserted into the right femoral vein in rabbits and the right jugular vein in guinea pigs. Sodium heparin (300–500 U/kg, i.v.) was used as an anticoagulant.

Surgical denervation of the heart and vagotomy: Five rabbits were anesthetized with urethane (1.0–1.2 g/kg, i.p.). Each rabbit was placed on its back on a table, and its chest was opened under artificial respiration. Nerves to the heart running in the connective tissue around the aorta, vena cava superior, pulmonary aorta and vein were cut according to the method of surgical denervation of the heart (30).

Vagotomy was carried out by cutting both vagus nerves at the neck.

Isolated guinea pig auricles: Four guinea pigs were killed by exsanguination under light ether anesthesia. The chests were opened, and the hearts were removed as quickly as possible, placed in a dissection bath filled with Ringer-Locke solution at room temperature and the atria were excised. The Ringer-Locke solution contained: 154 mM NaCl, 5.6 mM KCl, 1.1 mM CaCl_2 , 6.0 mM NaHCO_3 , 5.6 mM glucose. The preparation was mounted in a bath containing Ringer-Locke solution aerated with 100% O_2 . The bath temperature was maintained at 30°C. One end of the auricles was fixed to a pin in the bath bottom by thread and the other connected to a force-displacement transducer (Nihon Kohden, SB-1T). The contractile force and the beating rate were registered simultaneously on a recorder (Hitachi, 056). When the contractile force and beating rate became stable after the equilibration period of 30 to 60 min, drugs were introduced into the bath solution in a cumulative fashion, and the effects of the drugs were recorded.

Isolated perfused guinea pig heart (Lan-gendorff's preparation): Fourteen guinea pigs were killed by exsanguination under light ether anesthesia, and the hearts were removed as quickly as possible. Then isolated perfused guinea pig heart preparations were prepared according to the usual method (31). The preparation was retrogradely perfused with Krebs-Henseleit solution having the following composition: 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl_2 , 1.2 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.2 mM

KH_2PO_4 , 25.0 mM NaHCO_3 , 11.1 mM glucose. The perfusate was oxygenated by a mixed gas of 95% O_2 and 5% CO_2 , and its temperature was maintained at $37 \pm 0.5^\circ\text{C}$. The perfusion was carried out at a constant flow rate of 1.5–2.0 ml/min using a pump (Mitsumi Science, SJ-1210). The contractile force was recorded with the force-displacement transducer connected to the apex of the heart through a thread. Heart rate was counted by the signal of the contractile force. The contractile force and heart rate were registered simultaneously on the recorder. When the contractile force and heart rate became stable in 30 to 60 min after setting up the preparation, the perfusion fluid was changed to fluid containing drugs, and then the effects of the drugs on the preparation were recorded.

Drugs: Because commercially available formalin contains methanol as a stabilizer, we prepared methanol-free formaldehyde by distilling hexamethylenetetramine (Wako Pure Chemical) with sulfuric acid. The concentration of formaldehyde (HCHO) in the distillate was determined by iodimetry (32). Other drugs used were urethane (Sigma Chemical), anesthetic ether (Showa Ether), sodium heparin (Novo), DL-propranolol hydrochloride (Sigma Chemicals), reserpine (Apoplone® Inj., Daiichi Seiyaku), atropine sulfate (Sigma Chemicals).

Analysis of data: Statistical significance of data was analyzed by Student's *t*-test, and data are presented as the mean $\pm$ S.E.

Results

Responses to intravenous injection of HCHO in anesthetized guinea pigs and rabbits

HCHO (0.2–0.4 mg/kg), when intravenously given, produced immediately noticeable reductions in heart rate, blood pressure and respiratory movement in the chest in anesthetized guinea pigs. HCHO (2–4 mg/kg) produced similar effects in rabbits, although the responses to HCHO were about ten times smaller in rabbits than in guinea pigs, when compared on the basis of dose (Fig. 1).

Effects of HCHO on isolated guinea pig auricles

Assuming that the total blood volume is 8% of the whole body weight, the theoretical con-

Fig. 1. Responses of the heart rate (HR), blood pressure (BP) and respiratory movement (RM) to i.v. injection of HCHO in anesthetized guinea pigs and rabbits. Control values in guinea pigs (0.2 mg/kg HCHO, i.v.: HR=233±9, BP=43±4; 0.3 mg/kg HCHO, i.v.: HR=237±9, BP=47±4; 0.4 mg/kg HCHO, i.v.: HR=234±7, BP=53±4) (n=6-19). Control values in rabbits (2 mg/kg HCHO, i.v.: HR=258±8, BP=76±5; 4 mg/kg HCHO, i.v.: HR=265±11, BP=80±3) (n=5-8). Each value represents the mean±S.E. HR: beats/min, BP: mmHg.

Fig. 2. Effects of HCHO on the beating rate and contractile force in isolated guinea pig auricles. Ringer-Locke solution, 30°C, O₂. ●—●: HCHO, 6.6×10⁻⁴ M (2×10⁻⁵ g/ml), control values (BR: 120±8, CF: 0.46±0.07); ○—○: HCHO, 3.3×10⁻³ M (10⁻⁴ g/ml), control values (BR: 107±15, CF: 0.43±0.02); △—△: HCHO, 6.6×10⁻³ M (2×10⁻⁴ g/ml), control values (BR: 114±7, CF: 0.46±0.08); ▲—▲: HCHO, 3.3×10⁻² M (10⁻³ g/ml), control values (BR: 105±1, CF: 0.47±0.09). Each value represents the mean±S.E. (n=4). BR: beating rate (beats/min). CF: contractile force (g).

centration of HCHO in the blood, when HCHO was intravenously administered at the doses of 0.2–0.4 mg/kg in guinea pigs is calculated to be 2.5–5×10⁻⁶ g/ml. In the isolated auricle preparation, HCHO even at the concentration of 10⁻⁴ g/ml did not affect the beating rate (Fig. 2). Increasing the concentration up to 10⁻³ g/ml produced a decrease in beating rate (Fig. 2). However, the

decrease did not reach statistical significance. The contractile force was clearly increased by HCHO in a concentration range of 2×10⁻⁵ to 2×10⁻⁴ g/ml. However, when the concentration of HCHO was increased to 10⁻³ g/ml, it produced a negative inotropic effect.

Effects of HCHO on isolated guinea pig perfused heart (Langendorff's preparation)

As shown in Fig. 3, in the isolated perfused

Fig. 3. Effects of HCHO on the heart rate and contractile force in isolated guinea pig perfused hearts (Langendorff's preparations). Krebs-Henseleit solution, 37°C, 95% O₂ + 5% CO₂. □—□: HCHO, 3.3×10^{-4} M (10^{-5} g/ml), control values (HR: 88 ± 16 , CF: 2.1 ± 0.8); ●—●: HCHO, 6.6×10^{-4} M (2×10^{-5} g/ml), control values (HR: 91 ± 9 , CF: 2.2 ± 0.5); ▲—▲: HCHO, 1.3×10^{-3} M (4×10^{-6} g/ml), control values (HR: 93 ± 8 , CF: 2.1 ± 0.4). Each value represents the mean $\pm$ S.E. ($n=4-10$). HR: heart rate (beats/min), CF: contractile force (g).

heart preparation, HCHO ($1-4 \times 10^{-5}$ g/ml) produced inconsistent changes in the heart rate, which was not significantly different from the control values, while HCHO significantly suppressed the contractile force at concentrations higher than 10^{-5} g/ml. The concentration of 2×10^{-5} g/ml was four to eight times as high as the theoretical blood concentration of HCHO ($2.5-5 \times 10^{-6}$ g/ml) calculated in guinea pigs that intravenously received HCHO at the doses of 0.2–0.4 mg/kg. Propranolol (1.9×10^{-5} M) did not significantly affect the decreased contractile force and the altered heart rate by HCHO (2×10^{-5} , 4×10^{-5} g/ml) (Fig. 4). Propranolol hardly affected the control value of heart rate, but depressed the contractile force by about 15% in Langendorff's preparations.

Effects of autonomic blockers and surgical denervation of the heart on responses to HCHO in anesthetized animals

(1) **Atropine and vagotomy:** Though the bradycardiac response to HCHO (4 mg/

kg, i.v.) was slightly inhibited by atropine (2 mg/kg, i.v.) and by a combination of atropine and vagotomy, the difference in the response before and after the vagolytic procedure of atropine and vagotomy was statistically insignificant in five rabbits. Similarly, the procedure did not significantly affect the fall of blood pressure induced by intravenously administered HCHO. Guinea pigs showed results similar to those in rabbits (Table 1).

(2) **Propranolol:** The bradycardiac response to HCHO (4 mg/kg, i.v.) was mostly abolished by propranolol (2 mg/kg, i.v.) in four rabbits. Though the fall of blood pressure induced by the intravenously administered HCHO tended to be decreased by propranolol, it was not entirely abolished (Fig. 5, Table 1). Guinea pigs showed results similar to those obtained in rabbits (Table 1). Propranolol had little effect on the resting level of blood pressure, but decreased the heart rate by about 50% in rabbits and guinea pigs.

(3) **Reserpine:** The decrease in the heart

Fig. 4. Effects of propranolol on the heart rate and contractile force induced by HCHO in isolated guinea pig perfused hearts (Langendorff's preparations). Krebs-Henseleit solution, 37°C, 95% O₂ + 5% CO₂. ●—●: HCHO, 6.6×10^{-4} M (2×10^{-5} g/ml), control values (HR: 105 ± 2 , CF: 2.0 ± 0.4); ○—○: Propranolol (1.9×10^{-5} M) + HCHO (6.6×10^{-4} M), control values (before propranolol, HR: 104 ± 3 , CF: 2.1 ± 0.1 ; after propranolol, HR: 102 ± 6 , CF: 1.8 ± 0.3); ▲—▲: HCHO, 1.3×10^{-3} M (4×10^{-5} g/ml), control values (HR: 99 ± 2 , CF: 2.1 ± 0.5); △—△: Propranolol (1.9×10^{-5} M) + HCHO (1.3×10^{-3} M), control values (before propranolol, HR: 101 ± 3 , CF: 2.0 ± 0.4 ; after propranolol, HR: 99 ± 3 , CF: 1.7 ± 0.3). Each value represents the mean $\pm$ S.E. (n=4). HR: heart rate (beats/min), CF: contractile force (g).

rate by HCHO (4 mg/kg, i.v.) was almost abolished by pretreatment (24 hr before the experiment) with reserpine (5 mg/kg, s.c.) in four rabbits. Though the decreased blood pressure by intravenously administered HCHO was apparently inhibited by reserpine, it still remained (Fig. 6). Furthermore, the bradycardiac response to HCHO in four reserpinized guinea pigs was almost abolished, like that in rabbits (data not shown).

(4) **Surgical denervation of the heart:** The decrease in the heart rate by HCHO (4 mg/kg,

i.v.) was almost abolished by surgical denervation of the heart in five rabbits. On the other hand, although the fall of blood pressure induced by intravenously administered HCHO was significantly decreased by the surgical denervation of the heart, it still persisted (Fig. 7, Table 1).

Discussion

In this study, we investigated the mechanism of the bradycardia induced by intravenously administered HCHO in anesthe-

Table 1. Effects of blockers and denervation on the decrease of the heart rate (HR) and blood pressure (BP) induced by i.v. injection of HCHO in anesthetized rabbits and guinea pigs

Treatment	Animal	Decrease of HR (beats/min) and BP (mmHg) induced by i.v. injection of HCHO						Number of experiments	
		Control		After treatment		Change (%)			
		Resting level	Decreasing level	Change (%)	Resting level		Decreasing level	Change (%)	
Atropine (2 mg/kg, i.v.)	Rabbit	HR	276±15	214±10	21.8±3.8	262±12	223±10	14.8±1.3	5
		BP	81±9	37±6	53.3±5.0	77±8	41±6	48.2±3.9	5
	Guinea pig	HR	267±9	218±13	18.5±3.8	267±15	239±17	11.0±2.3	5
		BP	48±7	38±5	19.6±2.0	46±6	39±5	15.0±2.4	5
Atropine (2 mg/kg, i.v.) +Vagotomy	Rabbit	HR	276±15	214±10	21.8±3.8	247±10	211±9	14.6±1.3	5
		BP	81±9	37±6	53.3±5.0	80±11	46±8	42.1±6.1	5
Propranolol (2 mg/kg, i.v.)	Rabbit	HR	249±10	195±14	21.9±3.3	142±8	136±9	4.5±1.8*	4
		BP	80±3	33±4	58.9±3.7	82±3	51±9	37.4±10.0	4
	Guinea pig	HR	221±11	191±9	13.5±1.1	104±5	101±5	2.9±0.6*	4
		BP	65±5	42±6	36.6±4.9	58±8	44±10	27.4±6.5	4
Denervation of the heart	Rabbit	HR	258±7	202±7	21.7±1.1	161±23	152±21	5.2±1.3*	5
		BP	61±5	24±2	59.8±4.0	38±4	24±3	36.9±3.4*	5

Rabbit: HCHO, 4 mg/kg, i.v.; Guinea pig: HCHO, 0.4 mg/kg, i.v. Each value represents the mean±S.E. *Significant difference from the control ($P<0.01$), Student's *t*-test.

Fig. 5. Effects of propranolol on the heart rate (HR), blood pressure (BP) and respiratory movement (RM) induced by HCHO in an anesthetized rabbit.

Fig. 6. Responses of the heart rate (HR), blood pressure (BP) and respiratory movement (RM) to i.v. injection of HCHO in a reserpinized rabbit. Reserpine, 5 mg/kg, s.c., 24 hr before the experiment.

Fig. 7. Effects of surgical denervation of the heart on the heart rate (HR) and blood pressure (BP) induced by HCHO in an anesthetized rabbit. HCHO was injected in about 15–20 minutes after the denervation.

tized guinea pigs and rabbits. A comparison of the bradycardiac response to HCHO in these animals revealed that guinea pigs were about ten times more sensitive to HCHO than rabbits. At the present time we can not explain this species difference in sensitivity to HCHO. Because of the high sensitivity to HCHO in guinea pigs, we employed mainly guinea pigs for the experiments using isolated heart preparations. The concentration (10^{-3} g/ml) at which HCHO clearly depressed the beating rate in isolated auricles was 200 to 400 times higher than the theoretical blood concentration of HCHO ($2.5-5 \times 10^{-6}$ g/ml) calculated from the dose at which HCHO significantly decreased the heart rate in anesthetized guinea pigs. Moreover, in isolated auricle preparations, the contractile force was obviously suppressed by HCHO at the high concentrations (10^{-3} g/ml), but was increased by HCHO at the concentrations (2×10^{-5} – 2×10^{-4} g/ml) less than 10^{-3} g/ml. The cause of the increasing response in contractile force to HCHO was not examined. However, this increasing response is apparently not due to the release of endogenous catecholamines, because propranolol did not affect the inotropic response in the isolated auricles and in the perfused hearts (data not shown). Further investigation is necessary to clarify this point. The concentration (2×10^{-5} g/ml) at which HCHO did not decrease the heart rate but suppressed the contractile force in isolated perfused hearts was four to eight times as high as the theoretical blood concentration of HCHO at which HCHO produced the bradycardiac effect in anesthetized guinea pigs. Moreover, propranolol had no significant effect on the suppression of the contractile force and the change of heart rate induced by HCHO (2×10^{-5} , 4×10^{-5} g/ml) in isolated perfused hearts. As mentioned above, although HCHO at low doses markedly inhibited the heart rate in anesthetized animals, HCHO at concentrations low enough correspond to the blood concentrations attainable in *in vivo* experiments did not inhibit cardiac function in isolated heart preparations. Only higher concentrations of HCHO produced the inhibitory response. Therefore, these results suggest that the bradycardiac response to HCHO seems

unlikely to be due to its direct effect on the heart.

Pretreatment with atropine and surgical vagotomy had no significant effect on the negative chronotropic response to intravenously given HCHO in anesthetized animals. However, propranolol almost blocked this response. This finding indicates that the bradycardiac response to HCHO is due to a decrease in sympathetic nervous activities rather than due to increase in parasympathetic nervous activities.

The negative chronotropic response to HCHO was also blocked by reserpine. This fact further supports the idea that the decrease in the heart rate induced by HCHO results from the influence of HCHO on the sympathetic nervous system, that is, inhibiting transmitter release at the adrenergic nerve endings.

Interruption of tonic influence from the central nervous system by acute surgical denervation in cardiac sympathetic nerves did abolish the negative chronotropic response to HCHO, suggesting that the possible site of interaction of HCHO with the sympathetic nerves may be located in the central nervous system, even if only partly.

Concerning the fall of blood pressure induced by HCHO in rabbits, pretreatment with propranolol or reserpine attenuated it, but did not entirely abolish it. As described in the previous paper (22, 25), the fall of blood pressure induced by intravenously administered HCHO is caused by a combination of the direct relaxation of vascular smooth muscle and the inhibition of the sympathetic nervous system. The fall in blood pressure observed in this experiment appears to be produced mainly by the relaxation of vascular smooth muscle.

These results suggest that the direct action of HCHO on the heart was negligible for inducing the negative chronotropic response in anesthetized animals. Furthermore, the negative chronotropic effect of HCHO in animals seems to be caused mainly by the inhibition of sympathetic nervous activity through the central nervous system.

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