Effects of adrenal demedullation combined with chemical sympathectomy on cold-induced responses of endocrine pancreas in rats

Y. Habara, T. Ohno, T. Yahata and A. Kuroshima

Department of Physiology, Asahikawa Medical College, Nishikagura, Asahikawa, 078-11 (Japan), and Division of Nutrition Physiology, Hokkaido Educational College, Hokumoncho 9, Asahikawa, 070 (Japan), November 13, 1981

Summary. Adrenal demedullation combined with chemical sympathectomy with 6-hydroxydopamine (ACS) lowered plasma glucagon and insulin levels in rats. Acute cold exposure increased plasma glucagon in both ACS and control rats, while it increased plasma insulin only in ACS rats. ACS rats responded to cold with a smaller increase in plasma glycerol and a more pronounced elevation of plasma free fatty acids.

Although it is well established that catecholamines play a major role in nonshivering thermogenesis in cold acclimation[1], a series of reports from our laboratory[2-8] has suggested that glucagon is closely related to the development and the maintenance of cold acclimation. Furthermore, glucagon secretion has been shown to be specifically involved in the metabolic responses to acute cold exposure[7]. Cold stress is known to be a powerful stimulus for the increased sympathetic nervous activity[9]. It has been also reported that the sympathetic nervous system modulates glucagon release from A cell of Langerhans islets[10]. In the present study the changes of plasma glucagon level as well as insulin due to acute cold exposure were investigated in the adrenal demedullated and chemical sympathectomized rat in order to know the relation between sympatho-adrenal system and glucagon secretion in the cold.

Materials and methods. Male rats of Wistar strain weighing about 180 g were used throughout the experiments. They were divided into 2 groups. Bilateral adrenal demedullation[11] and chemical sympathectomy[12] with 6-hydroxydopamine (ACS) were performed in 1 group of rats (ACS group), and sham operation and injection of vehicle were given to the other group (SV group).

They were kept at 25±1 °C under artificial illumination from 07.00 to 19.00 h and allowed free access to commercial rat chow (Oriental MF, Oriental Yeast Co., Ltd) and tap water.

50 mg 6-hydroxydopamine (6-OHDA, Sigma Chem. Co., Saint Louis) dissolved in 1 ml of acidified saline (1% ascorbic acid in 0.9% NaCl) was s.c. injected in a dose of 50 mg 6-OHDA/kg b.wt every 4th day for 3 weeks[12]. 48 h after the last injection and in an 18-h fasted state the animals were subjected to acute cold exposure at −5 °C for 150 min. Blood samples were collected by decapitation, and hematocrit, blood glucose[13], glycerol[14] and free fatty acids (FFA)[15] were measured. Plasma glucagon and insulin levels were determined by radioimmunoassay technique (Glucagon Kit Daiichi and Insulin Kit Daiichi, respectively: Daiichi Radioisotope Institute, Tokyo). The statistical significance of the results was assessed by Student's t-test.

Results and discussion. The initial body weight was 186±3.5 g in the SV group and 185±3.9 g in the ACS group thus, there was not a significant weight difference between the groups. It was 246±3.3 g and 224±3.5 g at the time of decapitation, respectively. The increment of body weight was significantly smaller in the latter group ($p < 0.001$).

Table 1 shows the cold-induced changes in the plasma glucagon and insulin levels in both groups. At 25 °C the plasma glucagon level of ACS group was significantly lower than that of the SV group. Since catecholamines are known to stimulate glucagon release directly from A cell of Langerhans islets[10], this decreased glucagon level may be caused by the suppressed sympatho-adrenal activity. We have previously suggested that glucagon is closely associated with cold acclimation possibly through its lipolytic as well as glycogenolytic action[2-8,16]. The significant increase in plasma glucagon in response to cold exposure was again confirmed in the present experiment. Although the cold-induced increase of glucagon level was significantly smaller in the ACS group ($p < 0.01$), the extent of increment of glucagon level in the ACS rat (difference±95% confidential limit = 103±30.5 pg/ml) was not significantly different from that in the SV one (145±45.5 pg/ml). This result would appear to indicate that the sympatho-adrenal system, at least in part, mediates the cold-induced release of glucagon, although the possibility of glucagon release independent of sympatho-adrenal system cannot be completely excluded as we have previously suggested[7]. Plasma insulin level was not affected by acute cold exposure in the SV group. In the ACS group, however, the plasma insulin

Table 1. Changes in plasma glucagon and insulin levels

	Glucagon (pg/ml)	Insulin (μU/ml)
I Sham-operated and vehicle-treated (SV) group		
Warm controls (25 °C) (10)	175± 7.5	24.7±1.81
Cold-exposed (−5 °C) (10)	320±20.3[b]	26.1±2.36
II Demedullated and 6-OHDA-treated (ACS) group		
Warm controls (25 °C) (9)	140±4.4[d]	13.6±1.70[d]
Cold-exposed (−5 °C) (9)	243±13.7[b,c]	20.4±1.55[a]

Each value represents the mean±SE. Number in parenthesis indicates the number of animals used. [a,b]Significantly different from the warm counterpart, $p < 0.01$ and 0.001, respectively. [c,d]Significantly different from sham-operatet and vehicle-treated group, $p < 0.01$ and 0.001, respectively.

Table 2. Changes in blood metabolites

	Hematocrit (%)	Glucose (mg/dl)	Glycerol (mM)	FFA (μEq./l)
I Sham-operated and vehicle-treated (SV) group				
Warm controls (25 °C) (10)	46.9±0.41	93.3±1.64	0.09±0.003	521±13.6
Cold-exposed (−5 °C) (10)	49.9±0.40[b]	76.8±1.30[b]	0.25±0.013[b]	809±48.6[b]
II Demedullated and 6-OHDA-treated (ACS) group				
Warm controls (25 °C) (9)	42.2±0.57[d]	89.1±2.07	0.09±0.013	446±25.7*
Cold-exposed (−5 °C) (9)	50.7±0.64[b]	75.3±3.84[a]	0.19±0.016[b,d]	1094±45.7[b,d]

Legends same as in table 1. *$p < 0.02$.

level was depressed but increased by acute cold exposure. It has been shown that catecholamines inhibit insulin release mediated by an α-receptor- and stimulate it by a β-receptor-mechanism. Furthermore, insulin release is known to be enhanced by glucagon[17]. The diminution of basal insulin level by ACS in warm controls may be caused by the elimination of stimulatory action of catecholamines through a β-receptor and by the decrease of plasma glucagon. The increased glucagon release induced by acute cold exposure, together with the lack of α-receptor-mediated inhibitory action of catecholamines due to ACS in cold-exposed ACS rats, may augment insulin release.

Table 2 shows the changes in the levels of blood metabolites. The significant increase in the hematocrit was observed when both groups of rats were exposed to a cold environment, indicating hemoconcentration due to cold exposure[18]. ACS caused a significant decrease in the hematocrit in the warm controls but did not affect the cold-induced increase in the hematocrit.

Acute cold exposure induced a significant decrease of blood glucose level, suggesting that plasma glucose was utilized as the energy source in the cold as previously reported[7]. Plasma glycerol was significantly elevated when the animals in both groups were acutely exposed to cold. However, the extent of increment was significantly smaller in the ACS group than in the SV one. Plasma FFA was also increased by acute cold exposure in both groups. Its increment in the ACS group, however, was significantly larger than in the SV one ($p < 0.001$). The change in plasma glycerol level is considered to be a better index of magnitude of overall lipolysis, since plasma FFA released together with glycerol is metabolized more rapidly than glycerol[19]. Therefore, the plasma glycerol level is believed to reflect a degree of magnitude of lipolysis, that is, an activation of lipolysis is accompanied by a corresponding increase in the plasma glycerol level. From the present results, it is suggested that mobilization as well as utilization of lipids is significantly suppressed in the cold-exposed ACS rats. Although there is no doubt that sympatho-adrenal system is important for the cold-induced responses of animals and for cold acclimation, the increase in the plasma glucagon level by cold exposure may be also closely associated, at least in part, with the cold-induced responses, especially nonshivering thermogenesis, through its lipolytic action.

1 Janský, L., Biol. Rev. *48* (1973) 85.
2 Kuroshima, A., and Doi, K., Experientia *32* (1976) 473.
3 Kuroshima, A., Ohno, T., and Doi, K., Experientia *33* (1977) 240.
4 Kuroshima, A., Doi, K., and Ohno, T., J. physiol. Soc. Jap. *39* (1977) 405.
5 Kuroshima, A., Doi, K., and Ohno, T., Life Sci. *23* (1978) 1405.
6 Kuroshima, A., Doi, K., and Ohno, T., Jap. J. Physiol. *29* (1979) 661.
7 Kuroshima, A., Yahata, T., and Ohno, T., Jap. J. Physiol. *31* (1981) 43.
8 Yahata, T., Ohno, T., and Kuroshima, A., Life Sci. *28* (1981) 2603.
9 Picotti, G.B., Carruba, M.O., Ravazzani, C., Cesura, A.M., Galva, D., and Da Prada, M., Eur. J. Pharmac. *69* (1981) 321.
10 Smith, P.H., and Madson, K.L., Diabetologia *20* (1981) 314.
11 Evans, G., Am. J. Physiol. *114* (1935) 297.
12 Mory, G., Nechad, M., Ricquier, D., and Hémon, Ph., Experientia suppl. *32* (1978) 113.
13 Roe, J.H., J. biol. Chem. *212* (1955) 335.
14 Laurell, S., and Tibbling, G., Clinica chim. Acta *13* (1966) 317.
15 Itaya, K., and Ui, M., J. Lipid Res. *6* (1965) 16.
16 Lefebvre, P., Biochem. Pharmac. *24* (1975) 1261.
17 Samols, E., Marri, G., and Marks, V., Lancet *2* (1965) 415.
18 Bass, D.E., and Henschel, A., Physiol. Rev. *36* (1956) 128.
19 Portet, R., Laury, M.C., Bertin, R., Senault, C., Hluszko, M.T., and Chevillard, L., Proc. Soc. exp. Biol. Med. *147* (1974) 807.

0014-4754/83/040399-02$1.50 + 0.20/0

Effect of dimethindene, an antihistaminic drug, on the transmembrane potentials of mammalian myocardium

J. Mészáros, E. Hlavacsek-Póka[1] and J. Szegi[2]

Department of Pharmacology, Medical University of Debrecen, H-4012 Debrecen (Hungary), March 26, 1982

Summary. Dimethindene (DMI) decreased the maximum rate of rise of action potential (AP) without changing the resting potential in cat ventricular myocardium. DMI abolished the histamine-induced slow APs in left atria but not in right ventricular papillary muscles of guinea-pig, suggesting that DMI blocked the histamine H_1-receptors.

DMI (Forhistal®, Fenistil®) is a well-known antihistaminic drug used in the treatment of different allergies[3]. This activity of DMI has been attributed to antagonize the effects of histamine at the H_1-receptors[4]. It was demonstrated that the drug exerted many other actions, such as induction of histamine release, decrease of arterial blood pressure and peripheral resistance in anesthetized dogs[5]. Recently, it has been shown that DMI has an antiarrhythmic activity[6].

The aim of the present work was to study the effect of DMI on the normal and slow APs of the mammalian myocardium.

Methods. Experiments were carried out on isolated right ventricular papillary muscle of cat and guinea-pig, and on left atrial muscle of guinea-pig. The animals were anesthetized with ether, and the muscles were dissected from the heart as quickly as possible and mounted in an organ chamber. The preparations were driven electrically at 2.0 or 0.5 Hz. The transmembrane potentials were recorded by means of conventional glass microelectrode technique[7].

The slow response APs were elicited with histamine (10^{-5} M)) or caffeine (2 mM) in partially depolarized (up to -40 mV) left atrial and right ventricular myocardium of guinea-pigs. The membrane was depolarized by means of elevated K^+ (26 mM)-Krebs solution (an isosmolar substitution of K^+ for Na^+)[8]. DMI (Fenistil®, Zyma-Biogal) was freshly dissolved and added to the organ chamber containing Krebs solution (composition in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, NaH_2PO_4 1.0, $MgCl_2$ 1.2, $NaHCO_3$ 24.9, glucose 11.5, which was gassed with 95% O_2 and 5% CO_2 and kept at 37 °C.

Results. In the 1st series of experiments, the effect of DMI was examined on normal APs in right ventricular papillary muscle of cat. Figure 1 shows the dose-dependent effect of DMI. The control APs obtained in 5 preparations had the following parametes: overshoot $+21.4 \pm 0.7$ mV, the maximum rate of rise of AP ($\dot{V}_{max}$) 128.3 ± 0.5 V/sec, duration at 50% repolarization 170.5 ± 0.9 msec. The resting potential was -79.3 ± 1.2 mV (means $\pm$SEM of 5 experiments). Application of DMI (5×10^{-6} M) decreased the $\dot{V}_{max}$ to a