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Study on Hypoglycemic Effects of Muscles of Pig (*Sus scrofa*), Chicken (*Gallus domesticus*) and Mackerel (*Scomber japonicus*)

REIKO MATSUDA and MICHINORI KUBO*

Faculty of Pharmaceutical Sciences, Kinki University,
3-4-1, Kowakae, Higashiosaka, Osaka 577, Japan

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The hypoglycemic activity of extracts from muscles of pigs (*Sus scrofa*; P), chickens (*Gallus domesticus*; C) and mackerel (*Scomber japonicus*; M) was examined. P exhibited an insulin-like activity on conversion of glucose to total lipids in rat free fat cells and an insulin secretion-promoting effect on perfused rat pancreas. C showed an insulin-like activity, an insulin secretion-promoting effect and an inhibitory effect on hepatic glycogenolysis. M proved to have a weak insulin-like activity, and a weak insulin secretion-promoting effect, but its inhibitory effect on hepatic glycogenolysis was strong.

After intraperitoneal administration of P, C and M, the blood glucose levels decreased in both normal mice and alloxan-induced diabetic mice.

Keywords—hypoglycemic effect; *Sus scrofa*; *Gallus domesticus*; *Scomber japonicus*; diabetes; insulin-like effect; insulin secretion; glycogenolysis

Introduction

The syndrome which is diagnosed under the name of “xiaōkě (in Chinese)” and “shokachi (in Japanese),” is found in the traditional Chinese system of medicine. It is known that shokachi syndrome is closely related to the syndrome of diabetes. Various crude drugs used for treatment of shokachi syndrome have been described in successive Chinese herbal and medical books. It is also mentioned that shokachi syndrome is improved by eating meat and fish products or by drinking soups made from such products. That is to say, in poultry part II of volume 48 of “ben-cao-gang-mu,”¹⁾ it is stated that the decoction of yellowish hen is used for treatment of shokachi syndrome with urinary frequency. The meats of hog, sheep and cattle are also listed as materials for treating shokachi in the beast part of volume 50.

In the preceding paper,²⁾ we have reported that hot water extracts of various edible meat and fish products had an insulin-like activity based on conversion of [2-³H]D-glucose to total lipids in rat free fat cells and promoted secretion of insulin from the isolated islets of hamster. Accordingly, substances possessing hypoglycemic effect appear to be present in the products tested.

This study was designed to examine the hypoglycemic activity of the extracts of muscles of pig (*Sus scrofa*), chicken (*Gallus domesticus*) and mackerel (*Scomber japonicus*), based on conversion of glucose to total lipids in rat free fat cells, promotion of insulin secretion in isolated perfused rat pancreas *in vitro*, and inhibition of glucose output through glycogenolysis in the perfused liver *in situ*. Furthermore, hypoglycemic activity of the extracts was investigated in alloxan-induced diabetic mice *in vivo*.

Materials and Methods

Materials—The musculus iliopsoas of pigs (*Sus scrofa* L. var. *domesticus* BRISS), the musculus thoracis

profundus of chickens (*Gallus domesticus* BRISS) and the musculus latero-dorsalis and the musculus carinatus dorsalis of mackerel (*Scomber japonicus* HOUTTUYN) were used. Each muscle used was a fresh one isolated quickly after sacrifice. The extracts of muscles were obtained according to the method of Ehira and Uchiyama.³⁾ Sliced muscle (1 g) was mixed with 5 ml of phosphate buffer ($I=0.05$, pH 7.5), homogenized twice for 90 s in a homogenizer and then centrifuged at 5000 g for 15 min to give a supernatant. This procedure was repeated once more. The combined supernatant was lyophilized. Ice-cooling was maintained throughout the extraction. The extracts were abbreviated as P (pig), C (chicken) and M (mackerel).

The protein contents assayed by Lowry's method of P, C and M were 398, 491 and 504 mg/g, respectively. In all experiments, concentration or dosage of P, C and M is given as protein.

The sources of chemicals and reagents were as follows: porcine insulin (Sigma Chemical Co.), tolbutamide (Sigma Chemical Co.), bovine serum albumin (Fraction V, Sigma Chemical Co.), collagenase (Type I, Worthington Biochemical Corp.), [2-³H]D-glucose (Amersham Laboratories), alloxan (Nakarai Chemicals Ltd.), epinephrine (Bosmin inj., Daiichi Seiyaku Co., Ltd.). The determination of glucose was performed by the mutarotase-glucose oxidase method⁴⁾ using Glucose C-Test Wako (Wako Pure Chemical Ind. Ltd.). The determination of insulin levels was performed by radioimmunoassay (RIA) using the IRI 'Eiken' radioimmunoassay kit (Eiken Chemical Co., Ltd.) and by enzyme immunoassay (EIA) using Mesa insulin test (MBL Co., Ltd.). In these assays, rat insulin (Novo Research Laboratory) was used as the standard.

Animals—Male mice of Jcl: ICR strain and male rats of Kwl: Wistar strain were used. The animals were housed in a room maintained at $23 \pm 1^\circ\text{C}$ and 60% relative humidity for 7 d before the start of the experiment, with free access to food and water.

Alloxan-induced diabetic mice were prepared by injecting alloxan at a dose of 60 mg/kg into the tail vein of mice (5 weeks of age, 24–27 g). Two days later, blood was drawn from the orbit, and the glucose level was determined. Mice with blood glucose levels exceeding 300 mg/dl were used as diabetic mice.

Insulin-like Activity on Conversion of [2-³H]D-Glucose to Total Lipids in Rat Free Fat Cells—The isolated fat cells were prepared by the collagenase method of Rodbell⁵⁾ from epididymal adipose tissue of rat (5 weeks of age, 120–150 g). The cell suspension in Krebs-Ringer-bicarbonate (KRB) buffer, containing 20 mM Hepes and 2% bovine serum albumin at pH 7.4 was allowed to stand for 30 min at 37°C . According to the method of Moody *et al.*,⁶⁾ the cell suspension (2×10^5 cells/ml) was incubated with 0.55 mM glucose, [2-³H]D-glucose (0.04 μCi) and a sample (10–200 $\mu\text{g/ml}$) for 2 h at 37°C . The incubation with each sample (50 $\mu\text{g/ml}$) was also performed in the presence of porcine insulin (10–100 $\mu\text{U/ml}$). Then 8 N H_2SO_4 (0.2 ml) and scintillation fluid were added to the cell suspension, and the radioactivity of the resulting toluene layer was measured with a scintillation counter. Insulin-like activity of each sample was calculated on the basis of the following formula.

$$\text{activity \%} = (B - A) / (A - C) \times 100$$

A: radioactivity of total lipids after incubation with buffer alone

B: radioactivity of total lipids after incubation with sample

C: radioactivity of total lipids before incubation

Promotion of Insulin Secretion from the Isolated Perfused Rat Pancreas—Perfusion of rat (10 weeks of age, 300–350 g) pancreas was performed according to the method of Seino *et al.*⁷⁾ All perfusions were conducted with KRB buffer containing 0.25% bovine serum albumin, 3.8% dextran and 5.5 mM glucose. The perfusion solution and each sample solution were passed through membrane filters of 0.2 μm . The perfusion solution was gassed with 95% O_2 –5% CO_2 and maintained at pH 7.4 and 37°C . The flow rate was kept constant at 2.0 ml/min. After an equilibration period (20 min), the pancreas was perfused for 20 min with the perfusion solution containing a sample (200 $\mu\text{g/ml}$), and then further perfused for 5 min with perfusion solution alone. The effluent from the portal vein was collected in test tubes at intervals of one minute, frozen immediately and stored at -20°C until assayed. Insulin contents were assayed by the RIA method.

Glucose Output in Perfused Rat Liver—Rats (230–250 g) of 8 weeks of age were used. Liver perfusion was performed according to the method of Kimura *et al.*⁸⁾ The KRB buffer was gassed with 95% O_2 –5% CO_2 and maintained at pH 7.4 and 37°C . The flow rate was kept constant at 25 ml/min. KRB buffer and sample solutions were passed through membrane filters of 0.2 μm . Each sample solution was introduced through the side arm pump into the KRB buffer from the beginning of perfusion. After 40 min, epinephrine was injected into the perfusion solution over a 20-min period by means of an injection pump to give a final concentration of 1×10^{-7} mol. Glucose content in the effluent from the inferior vena cava was assayed by means of the mutarotase-glucose oxidase method.

Blood Glucose Levels in Normal Mice and Alloxan-Induced Diabetic Mice—Either normal mice (22–24 g) of 5 weeks of age or alloxan-induced diabetic mice at 6 d after alloxan induction were used. Each sample was administered intraperitoneally at a dose of 5 or 20 mg/kg. Blood was drawn from the orbit just before and 2, 4, 8 and 12 h after administration of the samples and the blood glucose levels were determined by means of the mutarotase-glucose oxidase method.

Glucose Tolerance Test—Either normal mice (22–24 g) of 5 weeks of age or alloxan-induced diabetic mice at

10 d after alloxan induction were fasted for 16 h and then orally given glucose at 2 g/kg. Blood was drawn from the orbit just before and 30, 60 and 120 min after administration of glucose and the blood glucose levels were determined. Serum insulin levels were measured in blood drawn just before and 30 min after administration of glucose. The ratio ($\Delta\text{EIA}/\Delta\text{BG}$) of increment of insulin (ΔEIA in ng/ml) to that of blood glucose (ΔBG in mg/dl) at 30 min after glucose load represented as the insulin secretion index.^{9,10} Each sample was administered intraperitoneally at a dose of 5 or 20 mg/kg 4 h before administration of glucose. Serum insulin levels were determined by the EIA method.

Epinephrine-Induced Hyperglycemic Mice—After 16 h of fasting, mice (22–24 g) of 5 weeks of age were used. Epinephrine was administered subcutaneously at the dose of 1 $\mu\text{g}/\text{kg}$. One hour later, blood was drawn from the orbit and the blood glucose levels were determined. Each sample was administered intraperitoneally at a dose of 5 or 20 mg/kg 4 h before s.c. administration of epinephrine.

Statistical Analysis—Statistical analysis was done by using either Student's *t*-test or the paired *t*-test.

Results

Insulin-like Activity of P, C and M on Conversion of $[2\text{-}^3\text{H}]\text{D}$ -Glucose to Total Lipids in Rat Free Fat Cells

As shown in Fig. 1, P, C and M exhibited concentration-dependent insulin-like activity in the conversion of $[2\text{-}^3\text{H}]\text{D}$ -glucose to total lipids. P and C were more potent than M.

The activity of P (50 $\mu\text{g}/\text{ml}$) together with 10–100 $\mu\text{U}/\text{ml}$ porcine insulin is shown in Fig. 2. The activities were additive at a low concentration (10 $\mu\text{U}/\text{ml}$) of porcine insulin but not at high concentrations (25–100 $\mu\text{U}/\text{ml}$). In the cases of C and M, the activity was similar to that of porcine insulin alone in this experiment.

Promoting Effects of P, C and M on Insulin Secretion from the Isolated Perfused Rat Pancreas

Glucose (16.7 mM), as the positive control, exhibited a strong biphasic insulin secretion-promoting effect. Tolbutamide (0.5 mM) exhibited a monophasic insulin secretion-promoting effect in the initial stage of perfusion. P and C at 200 $\mu\text{g}/\text{ml}$ showed a similar pattern of insulin

Fig. 1. Insulin-like Activity of P, C and M on Conversion of $[2\text{-}^3\text{H}]\text{D}$ -Glucose to Total Lipids in Isolated Rat Adipocytes

P, —●—; C, —■—; M, —▲—. Activity (%) was represented by the following formula: activity % = $(B - A)/(A - C) \times 100$ (*A*, radioactivity of total lipids after incubation with buffer alone; *B*, radioactivity of total lipids after incubation with sample; *C*, radioactivity of total lipids before incubation). Each value represents the mean $\pm$ S.E. of 4 experiments.

Fig. 2. Effects of P on Conversion of $[2\text{-}^3\text{H}]\text{D}$ -Glucose to Total Lipids by Insulin in Isolated Rat Adipocytes

Insulin, —○—; insulin + P (50 $\mu\text{g}/\text{ml}$), —●—. Activity (%) was represented by the following formula: activity % = $(B - A)/(A - C) \times 100$ (*A*, radioactivity of total lipids after incubation with buffer alone; *B*, radioactivity of total lipids after incubation with insulin or insulin + P; *C*, radioactivity of total lipids before incubation). Each value represents the mean $\pm$ S.E. of 4 experiments. Significantly different from the same concentration of insulin, a) $p < 0.05$, b) $p < 0.01$.

Fig. 3. Effects of P, C, M, Glucose and Tolbutamide on Promotion of Insulin Secretion from the Isolated Perfused Rat Pancreas (Glucose 5.5 mM)

P (200 μ g/ml), —●—; C (200 μ g/ml), —■—; M (200 μ g/ml), —▲—; glucose (16.7 mM), —; tolbutamide (0.5 mM), ----. Each value represents the mean of 3 experiments.

secretion promoting effect, the effect being stronger with P. M (200 μ g/ml) had very little or no effect (Fig. 3).

Effects of P, C and M on Glucose Output in Perfused Rat Liver

As shown in Table I, the glucose output at 5 min after perfusion in the control was $20.01 \pm 0.86 \mu\text{mol}/5 \text{ min/g}$ wet tissue, and the output decreased thereafter. Porcine insulin at 1 mU/ml significantly inhibited the glucose output compared with that of the control during perfusion. M at 200 μ g/ml significantly inhibited the glucose output at 25 and 30 min after perfusion. In the cases of P and C (200 μ g/ml), inhibition of glucose output was observed at 20 and 30 min after perfusion, respectively. M concentration-dependently inhibited the total glucose output during perfusion for 30 min (Fig. 4).

Changes in the effluent glucose after addition of epinephrine with or without test samples and porcine insulin are shown in Fig. 5. Porcine insulin (1 mU/ml) inhibited the glucose output due to addition of epinephrine at all time points. M also showed significant inhibition at 200 μ g/ml. The inhibitory effect of C at 200 μ g/ml was weak, while P at 200 μ g/ml had no effect.

Effects of P, C and M on Blood Glucose Levels in Normal Mice and Alloxan-Induced Diabetic Mice

Sequential changes in the blood glucose levels after intraperitoneal administration of saline (control), P, C, M and tolbutamide in normal mice are shown in Table II. In the saline group, the blood glucose levels tended to rise during the observation period. The blood glucose levels were significantly decreased up to 4 h after administration at 20 mg/kg of tolbutamide. Blood glucose levels in the P, C and M 5 and 20 mg/kg groups were increased somewhat at 2 h after administration, but decreased at 4 h, except in the M group. Even at 8 h after administration, blood glucose levels were still significantly lower in the C 20 mg/kg group and in the M 5 and 20 mg/kg groups.

Sequential changes in the blood glucose levels after intraperitoneal administration of saline (control), P, C, M and tolbutamide in alloxan-induced diabetic mice are shown in Table III. In the saline group, the blood glucose levels showed no change. In the porcine insulin (5 U/kg, s.c.) group, the blood glucose levels were decreased at 2 h after administration to about

TABLE I. Effects of P, C, M and Insulin on Glucose Output in Perfused Rat Liver

Treatment	Concentration ($\mu\text{g/ml}$)	No. of livers	Glucose production ($\mu\text{mol/g/5 min}$)					
			5	10	15	20	25	30 (min)
Control		9	20.01 ± 0.86	11.69 ± 1.04	10.96 ± 1.04	9.35 ± 0.81	8.21 ± 0.44	7.16 ± 0.47
P	200	5	16.11 ± 3.74	12.49 ± 3.41	10.87 ± 2.90	6.88 ± 0.83	6.18 ± 0.85	5.02 ± 0.56^a
C	200	5	16.01 ± 3.23	13.72 ± 2.03	10.26 ± 1.68	6.48 ± 0.55^a	6.37 ± 0.96	5.42 ± 0.71
M	200	5	15.65 ± 4.18	13.29 ± 3.47	10.74 ± 0.52	7.11 ± 1.02	5.45 ± 0.75^a	4.76 ± 0.46^a
Porcine insulin	1 (mU/ml)	3	12.49 ± 0.67^c	11.31 ± 0.01^b	8.90 ± 0.53	7.32 ± 0.14^a	6.29 ± 0.28^a	5.19 ± 0.63^a

Each value represents the mean $\pm$ S.E. Significantly different from the control at the same time, a) $p < 0.05$, b) $p < 0.01$, c) $p < 0.001$.

Fig. 4. Effect of M on Glucose Output in Perfused Rat Liver

Each value represents the mean $\pm$ S.E. of 6 experiments on total glucose output for 30 min after the beginning of perfusion. Significantly different from buffer alone, a) $p < 0.05$.

Fig. 5. Effects of P, C, M and Insulin on Glucose Output Induced by Epinephrine in Perfused Rat Liver

Control ($n=9$), —○—; P (200 $\mu\text{g/ml}$, $n=5$), —●—; C (200 $\mu\text{g/ml}$, $n=5$), —■—; M (200 $\mu\text{g/ml}$, $n=5$), —▲—; insulin (1 mU/ml, $n=3$), ----. Activity (%) shows changes in the effluent glucose after addition of epinephrine. Each value represents the mean $\pm$ S.E. Significantly different from the control at the same time point, a) $p < 0.05$, b) $p < 0.01$.

1/2 of the level observed before administration, but had largely recovered at 4 h. In the group treated with tolbutamide at 50 mg/kg, the blood glucose levels were lowest at 2 h, and showed a gradual recovery thereafter, but the effect was significant even 8 h after administration. Blood glucose levels in the P, C and M 5 and 20 mg/kg groups were all decreased at 2 h. The effect was still significant at 8 h in the P 5 mg/kg group, C 5 and 20 mg/kg groups and M 20 mg/kg group.

Effects of P, C and M on Glucose Tolerance Test

The changes in blood glucose levels and insulin secretion indexes obtained in the glucose tolerance test of normal mice are shown in Table IV. The tested samples and tolbutamide did not show any inhibitory effect on the rise of blood glucose levels at 30, 60 and 120 min after a glucose load. P at 20 mg/kg resulted in a significantly high value of insulin secretion index.

The differences between blood glucose levels before and after administration of glucose in

TABLE II. Effects of P, C, M and Tolbutamide on Change of Blood Glucose in Normal Mice

Treatment	Dose (mg/kg)	No. of mice	Route	Increase (%) of blood glucose ^{a)}			
				2	4	8	12 (h)
Control (saline)		12	i.p.	10.43 ± 4.74	11.32 ± 5.33	4.13 ± 3.92	17.86 ± 3.73
P	5	11	i.p.	4.88 ± 2.05	6.29 ± 3.52	2.20 ± 5.35	13.74 ± 2.51
	20	12	i.p.	7.65 ± 3.60	-3.97 ± 3.74 ^{b)}	0.62 ± 3.50	17.72 ± 4.61
C	5	11	i.p.	3.58 ± 3.76	-8.43 ± 6.06 ^{b)}	-4.68 ± 4.12	6.33 ± 4.57
	20	11	i.p.	0.01 ± 2.70	-8.99 ± 3.23 ^{c)}	-9.41 ± 2.94 ^{b)}	9.14 ± 3.06
M	5	11	i.p.	5.10 ± 3.26	-1.25 ± 4.26	-6.37 ± 2.78 ^{b)}	8.96 ± 3.00
	20	11	i.p.	5.33 ± 3.01	-4.27 ± 4.78 ^{b)}	-8.22 ± 3.60 ^{b)}	10.00 ± 2.96
Tolbutamide	20	12	i.p.	-5.72 ± 3.20 ^{b)}	-10.39 ± 3.85 ^{c)}	-6.34 ± 3.36	8.42 ± 3.40

a) Against blood glucose level just before administration of samples. Each value represents the mean ± S.E. Significantly different from the control at the same time, b) $p < 0.05$, c) $p < 0.01$.

TABLE III. Effects of P, C, M, Tolbutamide and Insulin on Changes of Blood Glucose in Alloxan-Induced Diabetic Mice

Treatment	Dose (mg/kg)	No. of mice	Route	Blood glucose (mg/dl)				
				Before	2	4	8	12 (h)
Normal		8		194 ± 6	194 ± 7	201 ± 7	178 ± 6	185 ± 5
Control (saline)		13	i.p.	542 ± 28	497 ± 27	511 ± 33	533 ± 32	580 ± 34
P	5	13	i.p.	558 ± 29	459 ± 24 ^{c)}	503 ± 28 ^{b)}	507 ± 39 ^{a)}	550 ± 38
	20	13	i.p.	546 ± 35	464 ± 38 ^{b)}	486 ± 45 ^{a)}	490 ± 45	542 ± 42
C	5	13	i.p.	531 ± 37	444 ± 34 ^{c)}	482 ± 38 ^{b)}	472 ± 39 ^{a)}	518 ± 47
	20	13	i.p.	588 ± 27	498 ± 30 ^{c)}	526 ± 33 ^{b)}	527 ± 37 ^{a)}	518 ± 47
M	5	13	i.p.	613 ± 23	580 ± 18 ^{c)}	526 ± 17 ^{a)}	579 ± 30	643 ± 34
	20	13	i.p.	574 ± 40	494 ± 38 ^{c)}	510 ± 34 ^{c)}	496 ± 39 ^{c)}	610 ± 50
Tolbutamide	50	10	i.p.	596 ± 39	437 ± 51 ^{b)}	494 ± 44 ^{b)}	505 ± 48 ^{b)}	547 ± 45
Porcine insulin	5 (U/kg)	10	s.c.	596 ± 31	314 ± 36 ^{c)}	555 ± 45	523 ± 47	579 ± 40

Each value represents the mean ± S.E. Significantly different by paired *t*-test, a) $p < 0.05$, b) $p < 0.01$, c) $p < 0.001$.

TABLE IV. Glucose Tolerance Test on Normal Mice

Treatment	Dose (mg/kg)	No. of mice	Route	Blood glucose (mg/dl)				Insulin secretion index ^{a)}
				Before	30	60	120 (min)	
Control (saline)		10	i.p.	75 ± 5	255 ± 16	219 ± 11	132 ± 7	19.56 ± 4.83
P	5	10	i.p.	51 ± 6 ^{b)}	235 ± 15	209 ± 6	112 ± 5	21.17 ± 5.71
	20	10	i.p.	51 ± 7 ^{b)}	264 ± 14	201 ± 11	124 ± 6	34.86 ± 4.91 ^{b)}
C	5	10	i.p.	56 ± 8	254 ± 11	213 ± 11	124 ± 6	19.28 ± 7.52
	20	10	i.p.	51 ± 3 ^{c)}	254 ± 13	210 ± 15	135 ± 7	19.27 ± 5.65
M	5	10	i.p.	57 ± 9	297 ± 26	262 ± 26	156 ± 12	19.20 ± 3.94
	20	9	i.p.	55 ± 6 ^{b)}	263 ± 22	215 ± 18	137 ± 9	17.83 ± 3.36
Tolbutamide	20	10	i.p.	47 ± 4 ^{c)}	315 ± 24	241 ± 17	133 ± 9	15.38 ± 3.36

a) The ratio (Δ EIA/ Δ BG) of increment of insulin (Δ EIA in ng/ml) to that of blood glucose (Δ BG in mg/dl) at 30 min after glucose load. Each value represents the mean ± S.E. Significantly different from the control, b) $p < 0.05$, c) $p < 0.01$.

alloxan-induced diabetic mice treated with the various agents are shown in Table V. The blood glucose levels reached a peak at 60 min in the saline (control) group. In the tolbutamide 50 mg/kg and P, C and M 5 and 20 mg/kg groups, the blood glucose levels reached a peak at

TABLE V. Effects of P, C, M, Tolbutamide and Insulin on Glucose Tolerance Test in Alloxan-Induced Diabetic Mice

Treatment	Dose (mg/kg)	No. of mice	Route	Increment of blood glucose (mg/dl) ^{a)}		
				30	60	120 (min)
Normal		8		112 ± 22	98 ± 14	11 ± 5
Control (saline)		12	i.p.	510 ± 26	517 ± 32	328 ± 29
P	5	13	i.p.	490 ± 25	444 ± 28	222 ± 35 ^{b)}
	20	13	i.p.	549 ± 43	487 ± 31	235 ± 25 ^{b)}
C	5	13	i.p.	529 ± 31	444 ± 44	269 ± 53
	20	11	i.p.	508 ± 35	484 ± 34	222 ± 34 ^{b)}
M	5	11	i.p.	555 ± 42	521 ± 26	296 ± 34
	20	12	i.p.	472 ± 51	415 ± 44	239 ± 41
Tolbutamide	50	9	i.p.	527 ± 27	481 ± 31	315 ± 37
Porcine insulin	5 (U/kg)	10	s.c.	512 ± 49	443 ± 57	278 ± 41

a) Against blood glucose levels before glucose load. Each value represents the mean ± S.E. Significantly different from the control at the same time, b) $p < 0.05$.

Fig. 6. Effects of P, C and M on Epinephrine-Induced Hyperglycemic Mice

Saline, ○; P, ●; C, ■; M, ▲. Each value represents the mean ± S.E. of blood glucose levels from 7 to 9 mice. Significantly different from the value for the saline-administered group, a) $p < 0.05$.

30 min. In the P 5 and 20 mg/kg and C 20 mg/kg groups, blood glucose levels were significantly decreased at 120 min compared with those of the control.

Effects of P, C and M on Epinephrine-Induced Hyperglycemic Mice

The hypoglycemic activities of P, C and M at the dosages of 5 and 20 mg/kg on epinephrine-induced hyperglycemic mice are shown in Fig. 6. C exhibited significant hypoglycemic activity at the dosages of 5 and 20 mg/kg. M was effective at 20 mg/kg only.

Discussion

Tsuda¹¹⁾ reported that in dietary therapy for diabetes, high-protein meals improved glucose tolerance in patients with mild diabetes as well as in hyperglycemic animals. In the previous paper,²⁾ we reported the existence of active constituents possessing hypoglycemic effects in these protein foods: the hot water extracts of edible meat and fish products, high protein foods, showed insulin-like effects on the conversion of glucose to total lipids in rat free fat cells and promoting activity on insulin secretion in the isolated islets of hamster. These

protein foods also contain small quantities of lipids and minerals, besides protein and water as main constituents. Among these constituents, it was confirmed that ashes obtained from the hot water extracts of these foods were ineffective in the *in vitro* biological tests. Thus, the minerals of the foods are not active components. In the present study, extracts of muscles of pig, chicken and mackerel were chosen as materials for experiments in order to elucidate the hypoglycemic activity of various protein foods. Protein is usually denatured on heating, and accordingly, the manipulation for obtaining the extracts was carried out under cool conditions. The resulting extracts contain approximate 50% of water-soluble proteins, commonly recognized as sarcoplasmic proteins.

P, C and M dose-dependently promoted conversion of glucose to total lipids in rat free fat cells, and P was most potent. In this *in vitro* experiment, when 50 $\mu\text{g}/\text{ml}$ of P and porcine insulin were simultaneously added into the medium, insulin-like activity of P and insulin activity were additive at a low concentration (10 $\mu\text{U}/\text{ml}$) of porcine insulin but not at higher concentrations (25–100 $\mu\text{U}/\text{ml}$). P strongly promoted insulin secretion from isolated rat pancreas, while C had weak activity, and M had no significant activity. These results were consistent with those of the *in vivo* glucose tolerance test as mentioned later, but differed from those in the previous report, in which the hot water extracts of the muscles of all three species showed significant activity. It is considered that these findings may be due to differences of experimental methods or of extraction procedures.

The glycogen metabolism in the liver and muscles is known to be one of the regulatory mechanisms maintaining homeostasis of the blood glucose levels. The effects of P, C and M on glycogenolysis in the liver were examined. M exhibited a significant inhibitory effect on glycogenolysis, but P and C had little effect.

It is well known that the activation of adenylyl cyclase and increases in adenosine 3',5'-cyclic monophosphate induced by epinephrine promote the decomposition of hepatic and muscular glycogen, and result in an increase of blood glucose levels. In the rat liver perfusion method, epinephrine promoted glycogenolysis and M inhibited glycogenolysis due to epinephrine. C also tended to inhibit it. C and M also inhibited epinephrine-induced hyperglycemia in mice. It was anticipated that C and M modify some process of glycogenolysis.

From these results obtained *in vitro* and *in situ*, it was assumed that P, C and M have hypoglycemic effects *in vivo*. Accordingly, we evaluated the hypoglycemic effects of P, C and M in normal mice and alloxan-induced diabetic mice. The experiments *in vivo* were performed by intraperitoneal injection because these samples contain about 50% proteins. P, C and M were found to decrease the blood glucose levels in normal mice and alloxan-induced diabetic mice. After a glucose load, P exhibited a significantly high value of insulin secretion index in normal mice. In the glucose tolerance test using alloxan-induced diabetic mice, P and C significantly promoted glucose clearance, and M showed a tendency to promote it.

Sodium phosphate, which is contained in the buffer solution used for extraction of muscles, did not exhibit any hypoglycemic activity in normal mice nor did it promote insulin secretion from isolated perfused rat pancreas in the same quantities as those in the extracts. Insulin content in P was 5.5 $\mu\text{U}/\text{mg}$ of porcine insulin, which did not influence blood glucose levels in the experimental models, and no insulin was detected in C or M.

Some active components possessing hypoglycemic activity appear to be present in the extracts of the muscles tested. Further studies are planned on the hypoglycemic effects of these extracts by oral administration, and to identify their active components.

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