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Comparative Biochemistry and Physiology, Part A 147 (2007) 156–164
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Central administration of vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide differentially regulates energy metabolism in chicks

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Received 14 September 2006; received in revised form 14 December 2006; accepted 14 December 2006
Available online 16 January 2007

Abstract

Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) are the members of the glucagon superfamily and bind to common receptors while PACAP also acts via the PACAP-specific receptor, PAC1. The aim of the present study was to investigate whether intracerebroventricular (ICV) injection of VIP and PACAP acts in a similar or different manner to affect body temperature and energy expenditure in the domestic chick. ICV injection of VIP did not significantly affect rectal temperature, but decreased energy expenditure. On the other hand, ICV injection of PACAP significantly increased both body temperature and energy expenditure. These specific actions of PACAP could be explained by an interaction with the PAC1 receptor, since they were partly, but not entirely, attenuated by PACAP (6–38), a PAC1 receptor antagonist. In addition, it was observed that central administration of both VIP and PACAP induced a reduction in respiratory quotient and increased plasma non-esterified fatty acid concentrations. This suggests that both peptides act centrally to regulate a catabolic response. In summary, brain VIP and PACAP both appear to exert generally catabolic effects on energy metabolism in the chick, but their influence on body temperature and glucose metabolism differs and their central effects do not appear to be mediated by the same receptors.

Keywords: Chicks; Energy expenditure; Intracerebroventricular injection; Pituitary adenylate cyclase-activating polypeptide; Plasma constituents; Rectal temperature; Vasoactive intestinal peptide

1. Introduction

Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) belong to the glucagon superfamily and bind to common receptors (Harmar et al., 1998). PACAP also binds to the specific PAC1 receptor (Harmar et al., 1998). These peptides regulate changes in physiological state through both peripheral and central pathways. Previous studies have revealed that central VIP and PACAP affect energy homeostasis in mammals. For example, intracerebroventricular (ICV) injection of PACAP inhibits feeding behavior of mice (Morley et al., 1992). VIP also exerts an anorexic effect in mammals (Woods et al., 1981). In addition, these peptides are involved in the control of body temperature and energy expenditure. Mice lacking the VPAC2 receptor, which is a common receptor for VIP and PACAP, showed an increase in basal metabolic rate when compared with their wild-type siblings (Asnicar et al., 2002). Moreover, since ICV injection of VIP decreases body temperature (following a slight increase in body temperature) in rats (Itoh and Hirota, 1982), central VIP appears to act as a hypothermic factor to decrease metabolic rate. On the other hand, ICV injection of PACAP increases body temperature in rats (Masuo et al., 1995; Pataki et al., 2000). In addition, mice lacking PACAP cannot survive in cold conditions (Gray et al., 2002), suggesting that PACAP acts as a hyperthermic factor and increases metabolic rate. These facts clearly demonstrate that although these peptides share a

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doi:10.1016/j.cbpa.2006.12.043

common receptor, they exert different physiological effects in mammals.

We have reported that both VIP and PACAP inhibit feeding behavior in chicks when administered centrally (Tachibana et al., 2003a), but induce their anorexigenic effects through different mechanisms (Tachibana et al., 2003b). We have demonstrated an interaction of both peptides with corticotropin-releasing factor (CRF) neurons (Tachibana et al., 2004b) and central CRF increases body temperature (Tachibana et al., 2004a) and energy expenditure (Tachibana et al., 2006) in chicks. These results suggest that VIP and PACAP are involved in the maintenance of energy balance by the brain. However, it is possible that the two peptides influence energy homeostasis in different ways. Therefore, in the present study, we investigated whether ICV injections of VIP and PACAP exerted similar or different effects on body temperature, energy expenditure, and respiratory quotient (RQ) in the chick. To investigate a possible influence of the peptides on glucose and lipid metabolism, we measured plasma concentrations of glucose (GLU), triacylglycerol (TG) and non-esterified fatty acids (NEFA) after ICV injection of VIP and PACAP. Finally, we determined the possible involvement of the PAC1 receptor in mediating the central effects of PACAP.

2. Materials and methods

2.1. Animals

Day-old male layer chicks (*Gallus gallus*, Julia strain) were purchased from a local hatchery (Murata Hatchery, Fukuoka, Japan) and kept in a room at 30 °C under continuous lighting. The birds were allowed free access to a commercial diet (Toyohashi Feed and Mills Co. Ltd., Aichi, Japan) and water except as noted elsewhere. Experimental procedures followed the guidance for Animal Experiments in Faculty of Agriculture and in the Graduate Course of Kyusyu University and the Law (No.105) and Notification (No.6) of the Government.

2.2. ICV injection

Rat VIP, rat PACAP-38 and PACAP (6–38) (all purchased from Peptide Institute, Osaka, Japan) were dissolved in a 0.1% Evans Blue solution prepared in a saline. The physiological action of mammalian VIP is weaker than that of chicken VIP in chicks (Nowak and Kuba, 2001). However, since the mammalian VIP could inhibit feeding behavior (Tachibana et al., 2003a,b, 2004b) and increase corticosterone release in chicks (Tachibana et al., 2004b) as shown in mammals, we decided that mammalian VIP is useful to investigate the effect of VIP and used mammalian VIP for the present study. The control group was injected with the same volume of this Evans Blue solution. The injection volume was 10 μl in all experiments.

ICV injection was conducted according to the method of Davis et al. (1979). Briefly, the head of the chick was inserted in an acrylic device which restrained the head and positioned a hole in a plate to lie immediately above the left lateral ventricle. A microsyringe was then inserted into the left lateral ventricle through the hole in the plate and the drug was injected. This method does not appear to induce physiological stress in the chick because ICV injection of saline solution, which was used as the control group in the present study, did not affect feeding behavior (Furuse et al., 1999) and corticosterone release (Saito et al., 2005) when compared to non-injected birds. Therefore, we did not anesthetize chicks for the injection and they were free to move and eat immediately after the injection. At the end of each experiment, chicks were sacrificed with an intraperitoneal overdose of sodium pentobarbital. Confirmation of drug injection was made by observation of the presence of Evans Blue dye in the lateral ventricle. The results obtained from chicks which did not have Evans Blue dye in the lateral ventricle were not used.

2.3. Experiment 1: effects of VIP and PACAP on rectal temperature

To determine rectal temperature, a 19-mm stainless sensor connected to a recorder (Thermalert TH-5, Physitemp Instruments Inc., New Jersey, USA) was inserted into the rectum. Briefly, each chick was removed from the cage and fixed with hand softly. The sensor was then inserted into the cloaca at a depth of 19 mm. The measurement of the rectal temperature was finished within 5 s, the chick was then returned to the cage.

After the measurement of the basal rectal temperature, each chick (6 days old) was ICV injected with 0 (control), 47 or 188 pmol VIP. The rectal temperature was then measured at 30 and 60 min after the injection. Food and water were not given to the chicks during the post-injection period. The number of chicks in each group was as follows: 0 pmol, 6; 47 pmol, 8; and 188 pmol, 6.

In the PACAP study, 5-day-old chicks were used and the experimental treatment was the same as used for the VIP study. The number of chicks in each group was as follows: 0 pmol, 7; 47 pmol, 8; and 188 pmol, 9.

2.4. Experiment 2: effects of VIP and PACAP on energy expenditure and RQ

To investigate energy expenditure, oxygen (O_2) consumption, carbon dioxide (CO_2) production and RQ were measured using an open-circuit calorimeter system (MK-5000RQ, Muromachi Kikai Co. Ltd., Tokyo, Japan). For the measurements, an acrylic chamber (150 mm × 150 mm × 150 mm) with a stainless steel grid floor was used. Fresh atmospheric air was drawn at a rate of 500 ml/min and then passed through O_2 and CO_2 detectors (MM202R, Muromachi Kikai Co., Ltd., Tokyo, Japan). The concentrations of these gases were recorded every 3 min. The analyzer was calibrated using primary gas standards of high purity (Sumitomo Seika Chemicals Co. Ltd., Osaka, Japan) every 1 h. Energy expenditure was calculated by the equation of Romijn and Lokhorst (1961) as follows: energy expenditure (kcal/min) = the volume of O_2 consumed (ml/

min)×3.871+the volume of CO_2 produced (ml/min)×1.194. The units for energy expenditure were converted to joules from calories by multiplying by 4.184 and the values obtained were normalized with the body weight.

Each chick (3 days old) was transferred to the test chamber for 1 h to allow acclimation to the chamber. The chick was then injected with 0 (control), 47 or 188 pmol VIP. The O_2 consumption and CO_2 production were measured for 1 h after the injection. During acclimation and experimental periods, chicks were not given access to food and water. The number of chicks in each group was 5.

The PACAP study was performed in exactly the same way as the VIP study. The number of chicks in each group was as follows: 0 pmol (control), 5; 47 pmol, 6; and 188 pmol, 6.

2.5. Experiment 3: effects of VIP and PACAP on locomotor activity

Before measurement of locomotor activity, chicks (4 days old) were placed into an acrylic behavioral cage (150 mm×250 mm×200 mm) for 1 h to accustom them to the experimental conditions. After this the chicks were ICV injected with 0 (control), 47 or 188 pmol VIP and then returned to the behavioral cage. Locomotor activity was measured for 1 h using infrared beam sensors (Neuroscience Inc., Tokyo, Japan) placed above the center of the monitoring cage. Food and water were not available to the chicks during the observation period. The number of chicks in each group was as follows: 0 pmol, 7; 47 pmol, 7; and 188 pmol, 5.

The PACAP study was done in exactly the same way as the VIP study but the number of chicks in each group was as follows: 0 pmol, 8; 47 pmol, 6; and 188 pmol, 7.

2.6. Experiment 4: effects of VIP and PACAP on plasma GLU, TG and NEFA concentrations

In the VIP study, chicks (6 days old) were ICV injected with 0 (control), 47 or 188 pmol VIP under ad libitum feeding conditions. At 30 min after the injection, blood was collected by heart puncture with a heparinized syringe. The blood was centrifuged at 9000×*g* and 4 °C for 4 min to obtain the plasma. Plasma GLU, TG and NEFA concentrations were measured using commercial kits (Wako Pure Chemical Industries, Osaka, Japan). The number of chicks in each group was as follows: 0 pmol, 9; 47 pmol, 6; and 188 pmol, 9.

The PACAP study was performed in the same way as the VIP study but the chicks used were 6 days old. The number of chicks in each group was as follows: 0 pmol, 7; 47 pmol, 6; and 188 pmol, 5.

2.7. Experiment 5: effect of PACAP (6–38) on PACAP-induced increase in body temperature

PACAP (6–38), a PAC1 receptor antagonist (Robberecht et al., 1992), was used to examine whether PACAP-induced increase in body temperature is mediated by the PAC1 receptor. The experimental procedures were the same as described for Experiment 1 but the chicks (5 days old) were ICV injected with saline (control), 188 pmol PACAP alone or 188 pmol PACAP plus 940 pmol PACAP (6–38). In PACAP plus PACAP (6–38) group, PACAP (6–38) was co-injected with PACAP. The dose of PACAP (6–38) did not affect the rectal temperature of chicks after it was injected ICV on its own (data not shown). The number of chicks in each group was as follows: saline, 9; PACAP alone, 8; and PACAP plus PACAP (6–38), 8.

2.8. Experiment 6: effect of PACAP (6–38) on PACAP-induced energy expenditure

Experimental procedures were the same as described for Experiment 2 but the chicks (3 days old) were injected with saline (control), 188 pmol PACAP alone or 188 pmol PACAP plus 940 pmol PACAP (6–38). In PACAP plus PACAP (6–38) group, PACAP (6–38) was co-injected with PACAP. The dose of PACAP (6–38) used did not affect O_2 consumption, CO_2 production, energy expenditure and RQ in chicks after ICV injection on its own (data not shown). The number of chicks in each group was as follows: saline, 6; PACAP alone, 5; and PACAP plus PACAP (6–38), 6. In the saline and PACAP plus PACAP (6–38) groups, 7 (n=3, at 36, 39, 42,

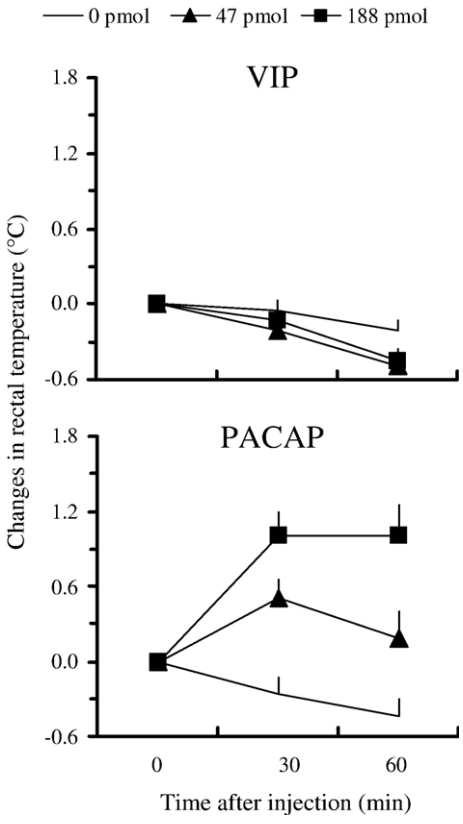

Fig. 1. Change in the rectal temperature after ICV injection of VIP or PACAP. In the VIP study, the number of chicks in each group was as follows: 0 pmol (control), 6; 47 pmol, 8; 188 pmol, 6. The number of chicks in each group of the PACAP study was as follows: 0 pmol (control), 7; 47 pmol, 8; and 188 pmol, 9. Data are expressed as means±S.E.M.

48, 54 and 57 min) and 1 ($n=1$, at 36 min) samples could not be measured for oxygen consumption owing to technical difficulties.

2.9. Statistical analysis

Data from Experiments 1, 2, 5 and 6 were statistically analyzed with repeated two-way analysis of variance (ANOVA) with respect to drug treatment and time. In Experiments 5 and 6, Tukey–Kramer test was used to compare between groups at each time point. Data from Experiments 3 and 4 were analyzed with one-way ANOVA and Fisher's PLSD test was then used as the post hoc test. Significant differences were set at $P<0.05$. Results are expressed as means ± S.E.M.

3. Results

3.1. Experiment 1: effects of VIP and PACAP on the rectal temperature

Fig. 1 shows the effect of ICV injection of VIP or PACAP on the rectal temperature of chicks. There was no significant difference [$F(2,17)=1.3$, $P=0.287$] in the rectal temperature immediately before the injection (0 min) between groups. ICV injection of VIP did not affect the rectal temperature [$F(2,17)=1.8$, $P=0.181$]. On the other hand, PACAP treatment significantly [$F(2,21)=14.0$, $P<0.01$] increased the rectal temperature. There was no significant difference [$F(2,21)=0.6$, $P=0.563$] in the rectal temperature immediately before the injection (0 min) between groups.

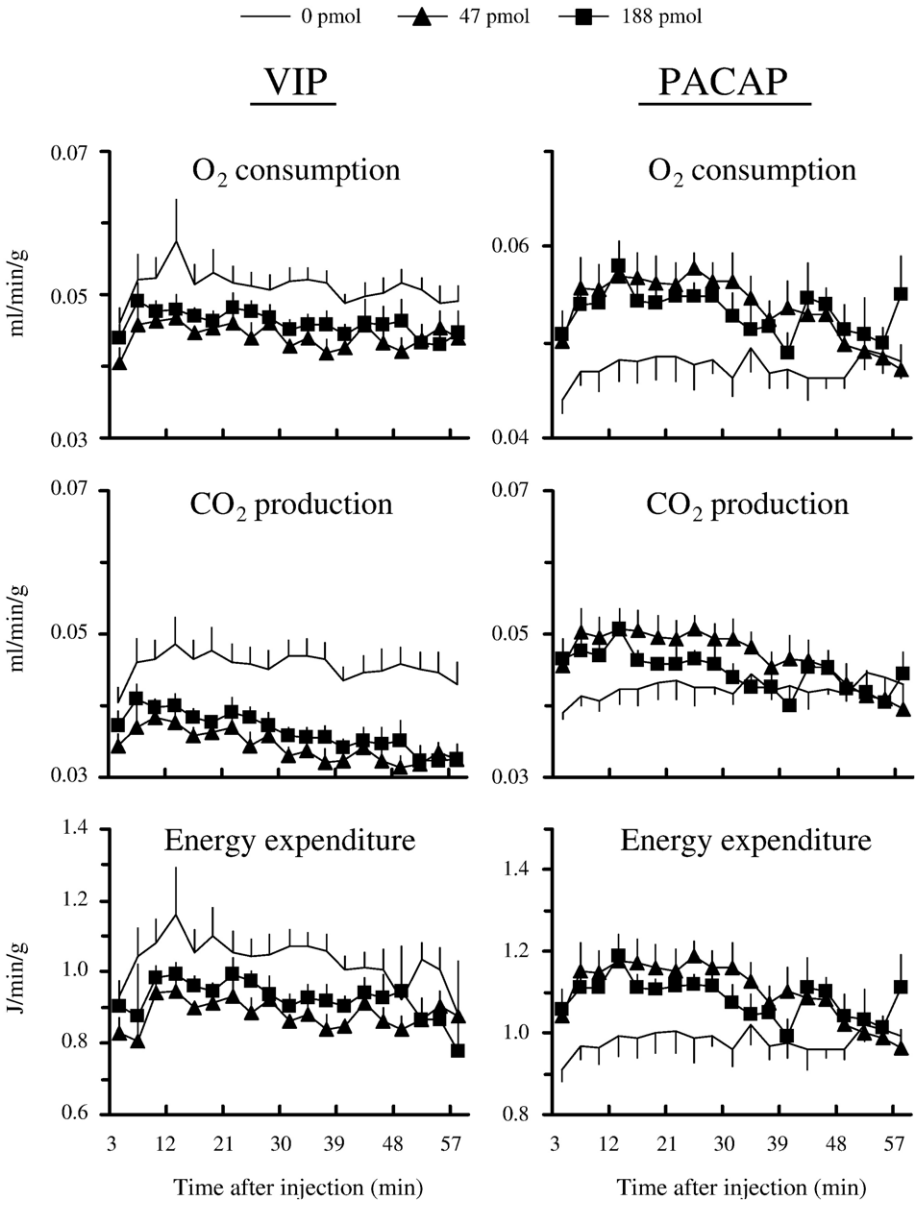

Fig. 2. Changes in O_2 consumption, CO_2 production and energy expenditure after ICV injection of VIP or PACAP. In the VIP study, the number of chicks in each group was 5. The number of chicks in each group of the PACAP study was as follows: 0 pmol (control), 5; 47 pmol, 6; and 188 pmol, 6. Data are expressed as means ± S.E.M.

3.2. Experiment 2: effects of VIP and PACAP on energy expenditure and RQ

The time-course of changes in O_2 consumption, CO_2 production and energy expenditure after the ICV injection of VIP or PACAP is shown in Fig. 2. VIP treatment significantly decreased O_2 consumption [$F(2,12)=4.7$, $P<0.05$], CO_2 production [$F(2,12)=9.8$, $P<0.01$] and energy expenditure [$F(2,12)=5.9$, $P<0.05$]. In the case of CO_2 production, the change was time-dependent because there was a significant interaction [$F(36,216)=1.6$, $P<0.05$] between time and treatment. In contrast to VIP, ICV injection of PACAP significantly increased O_2 consumption [$F(2,14)=4.0$, $P<0.05$] and energy expenditure [$F(2,14)=3.8$, $P<0.05$] but did not influence CO_2 production.

Fig. 3 shows the effect of ICV injection of VIP or PACAP on RQ. RQ was significantly [$F(2,12)=22.4$, $P<0.01$] decreased after the injection of VIP. There was a significant [$F(36,216)=5.9$, $P<0.01$] interaction between VIP treatment and time, indicating that the effect of VIP was time-dependent. The PACAP treatment also significantly [$F(2,14)=1.4$, $P<0.05$] decreased RQ. The decrease in RQ was time-dependent because a significant interaction [$F(36,252)=3.0$, $P<0.01$] between time and treatment was observed.

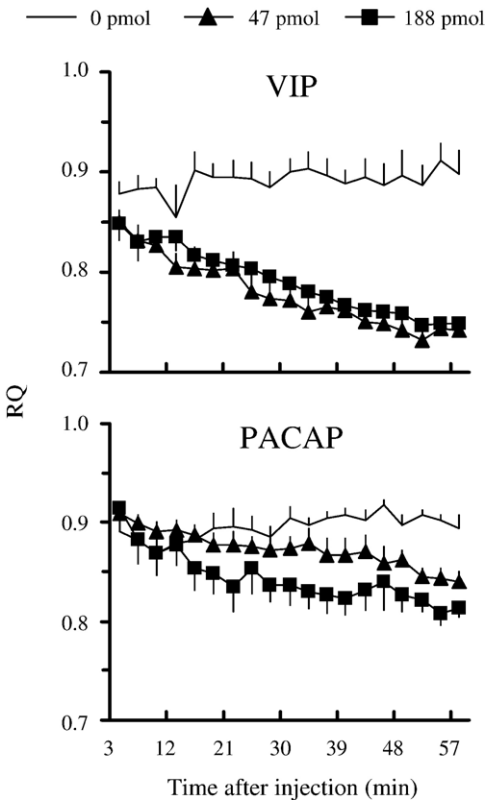

Fig. 3. Change in RQ after ICV injection of VIP or PACAP. In the VIP study, the number of chicks in each group was 5. The number of chicks in each group of the PACAP study was as follows: 0 pmol (control), 5; 47 pmol, 6; and 188 pmol, 6. Data are expressed as means±S.E.M.

3.3. Experiment 3: effects of VIP and PACAP on locomotor activity

Locomotor activity was not significantly affected by ICV injection of VIP [$F(2,16)=0.5$, $P=0.608$] or PACAP [$F(2,18)=1.5$, $P=0.240$] (data not shown).

3.4. Experiment 4: effects of VIP and PACAP on plasma GLU, TG and NEFA concentrations

Fig. 4 shows the plasma GLU, TG and NEFA concentrations after the ICV injection of VIP or PACAP. ICV injection of VIP significantly affected plasma GLU [$F(2,21)=3.7$, $P<0.05$] and NEFA concentrations [$F(2,21)=29.1$, $P<0.01$]. The post hoc test revealed that VIP significantly decreased and increased plasma GLU and NEFA concentrations, respectively. There was no significant difference in plasma TG concentration [$F(2,21)=2.0$, $P=0.165$]. On the other hand, ICV injection of PACAP significantly affected only plasma NEFA [$F(2,15)=5.5$, $P<0.05$] concentrations while GLU was not affected [$F(2,15)=3.1$, $P=0.075$] and TG [$F(2,15)=3.2$, $P=0.071$] was not affected. High dose of PACAP significantly increased plasma NEFA concentration.

3.5. Experiment 5: effect of PACAP (6–38) on PACAP-induced increase in body temperature

Fig. 5 shows the effect of PACAP (6–38) on PACAP-induced increase in the rectal temperature. There was no significant difference [$F(2,22)=0.5$, $P=0.603$] in the rectal temperature immediately before the injection (0 min) between groups. These treatments significantly [$F(2,22)=9.1$, $P<0.01$] affected the rectal temperature. Tukey–Kramer test revealed that the PACAP treatment alone significantly increased the rectal temperature as shown in Experiment 1, but the effect was significantly attenuated by PACAP (6–38) at 30 and 60 min after the injection.

3.6. Experiment 6: effect of PACAP (6–38) on PACAP-induced energy expenditure

Fig. 6 shows the effect of PACAP (6–38) on PACAP-induced changes in O_2 consumption, CO_2 production and energy expenditure. These treatments significantly affected O_2 consumption [$F(2,10)=6.7$, $P<0.05$] and energy expenditure [$F(2,10)=6.2$, $P<0.01$]. O_2 consumption, CO_2 production and energy expenditure were increased by ICV injection of PACAP alone as observed in Experiment 2. Values for these variables were comparable between the saline and PACAP plus PACAP (6–38) groups indicating that the effects of PACAP were attenuated by co-administration of PACAP (6–38). In fact, Tukey–Kramer test revealed that PACAP significantly increased O_2 consumption (at 3, 6, 9, 12, 27, 42, 51 and 57 min) and energy expenditure (at 3, 6, 9, 12 and 51 min) while the values are comparable between the control and PACAP plus PACAP (6–38) groups.

Fig. 7 shows the effect of PACAP (6–38) on the PACAP-induced change in RQ. RQ was not significantly [$F(2,10)=3.1$,

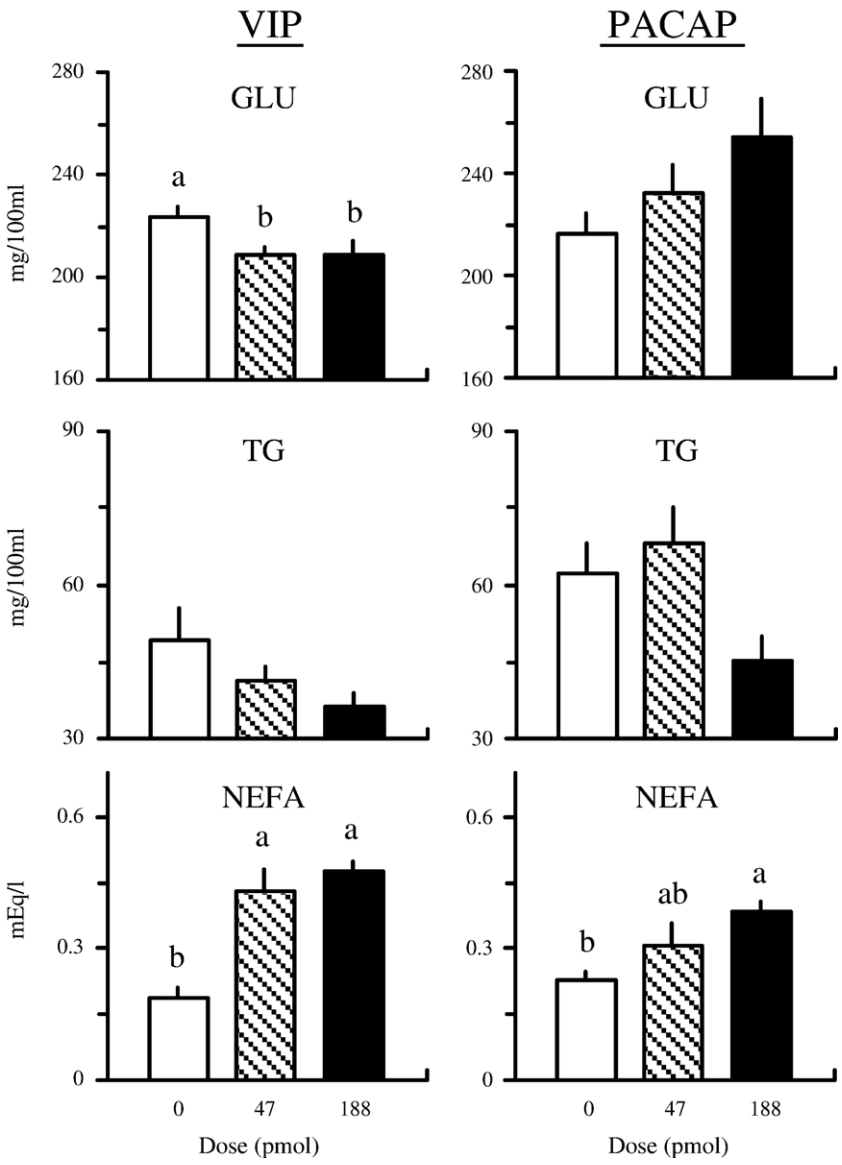

Fig. 4. Effect of ICV injection of VIP or PACAP on plasma GLU, TG and NEFA concentrations. In VIP study, the number of chicks in each group was as follows: 0 pmol (control), 9; 47 pmol, 6; and 188 pmol, 9. The number of chicks in each group of PACAP study was as follows: 0 pmol (control), 7; 47 pmol, 6; 188 pmol, 5. Data are expressed as means±S.E.M. Groups with different letters are significantly different ($P<0.05$).

$P=0.089$] affected by these treatments. ICV injection of PACAP alone lowered RQ as noted in Experiment 2, but the effect was tended to be negated by PACAP (6–38) treatment. Indeed, Tukey–Kramer test revealed that RQ in PACAP alone group was significantly lower than the control group at 48–57 min while there were no significant difference between the control and PACAP plus PACAP (6–38) group.

4. Discussion

We demonstrated here that ICV injection of VIP did not affect rectal temperature in chicks (Fig. 1) although there was a tendency for it to be decreased. This result is different from that obtained in a mammalian study in which ICV injection of VIP rapidly increased body temperature followed by decrease in body temperature in rats (Itoh and Hirota, 1982). On the other hand, ICV injection of PACAP did induce increase in body temperature in the chick (Fig. 1) as shown in mammals (Masuo et al., 1995; Pataki et al., 2000). The effect of PACAP on body temperature therefore appears to be conserved between birds and mammals. In rats, however, ICV injection of 55 pmol PACAP did not affect the body temperature (Pataki et al., 2000) while 47 pmol PACAP was sufficient to increase body temperature in chicks. In addition, 220 pmol PACAP induced increase in body temperature at 2 h after the injection (Pataki et al., 2000), although the effect was observed at 30 min in the present study. Although PACAP induces increase of body temperature in both chicks and rodents, the sensitivity and pattern would be different.

Since PACAP can bind to the VIP receptors VPAC1 and VPAC2, the inability of VIP to influence rectal temperatures appears to contradict the result obtained with PACAP. However,

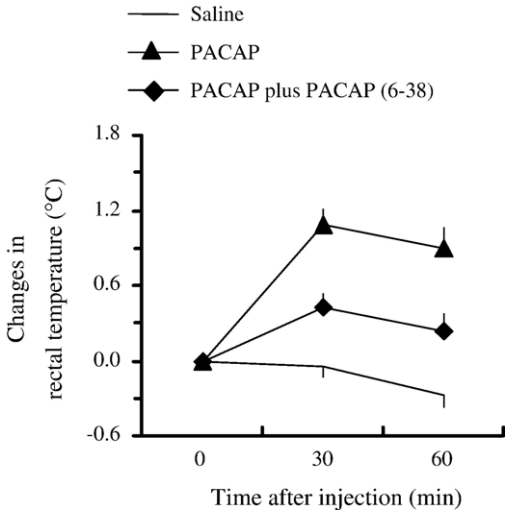

Fig. 5. Effect of ICV co-injection of PACAP (6–38) on PACAP-induced hyperthermia. The doses of PACAP and PACAP (6–38) were 188 and 940 pmol, respectively. The number of chicks in each group was as follows: saline (control), 9; PACAP alone, 8; and PACAP plus PACAP (6–38), 8. Data are expressed as means±S.E.M.

this could be explained if the effect of PACAP is mediated by the PAC1 receptor, and not via the VIP receptors, as suggested by the fact that ICV co-injection of PACAP (6–38) significantly attenuated the effects of PACAP (Fig. 5). The difference in the effects between VIP and PACAP would be due to the receptors. An alternative explanation may be related to the fact that mammalian VIP was used in the present study. The amino acid sequences of chicken PACAP, and of the PAC1 receptor, show high homologies to their mammalian counterparts (97% and about 82%, respectively) (Peeters et al., 1999; Sherwood et al., 2000) and the functions of the PAC1 receptor appear to be well conserved among vertebrate species. In contrast, the amino acid sequence similarities of chicken VIP and the VPAC receptors to mammalian sequences are 86% and about 60%, respectively (Sherwood et al., 2000; Kansaku et al., 2001). These comparatively low sequence similarities are likely to explain the fact that the ability of mammalian VIP to stimulate cAMP accumulation in the chick brain is weaker than that of chicken VIP (Nowak and Kuba, 2001). We have not yet directly compared the efficacy of chicken and mammalian VIPs on feeding behavior in chicks. However, we have demonstrated that mammalian VIP inhibits food intake in the chick (Tachibana et al., 2003a,b, 2004b) and, as discussed below, have shown an influence of VIP on energy expenditure, RQ, and plasma metabolites in the present study. These findings indicate that mammalian VIP is bioactive in the chick. Future investigations using chicken VIP are required to clarify the role of the peptide in the regulation of body temperature in chicks. However, the findings of the present study suggest that the regulation of body temperature by VIP may differ between chicks and mammals. Neonatal chicks less than 1 week after hatching is not fully developed their ability to keep body temperature (Hirabayashi et al., 2005). This might be the alternative reason why VIP did not affect the rectal temperature of chicks in the present study.

In contrast to its effects on rectal temperature, ICV injection of VIP induced a decrease in energy expenditure (Fig. 2), suggesting that central VIP acts as an inhibitor of energy expenditure in chicks. This hypothesis is supported by a mammalian study in which inactivation of the VPAC2 receptor induced increased basal metabolic rate in mice (Asnicar et al., 2002). In contrast, PACAP increased energy expenditure in chicks (Fig. 2) and this effect appeared to be mediated by the PAC1 receptor because PACAP (6–38) cancelled the effect of PACAP (Fig. 6). In rats, ICV injection of PACAP is reported to increase locomotor activity (Masuo et al., 1995). However,

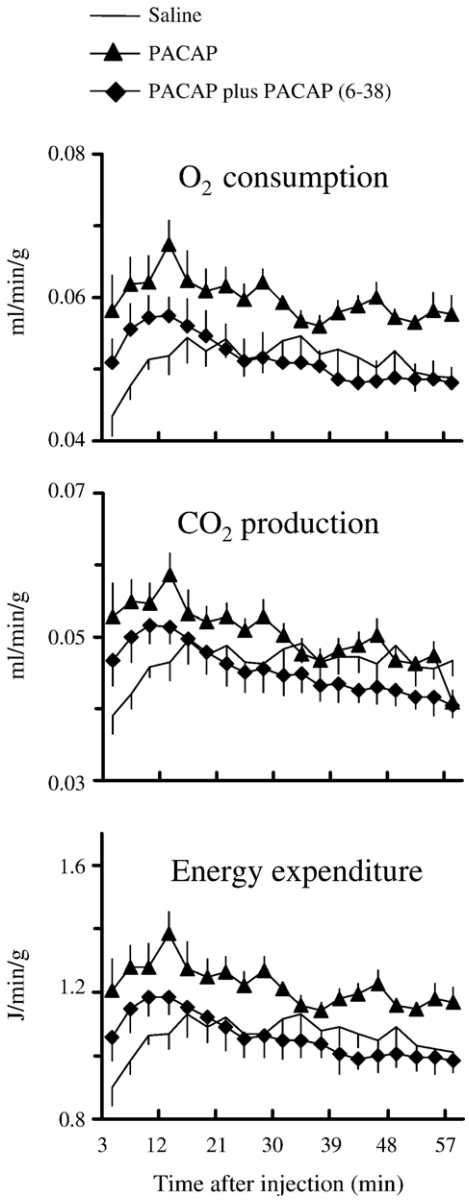

Fig. 6. Effect of ICV co-injection of PACAP (6–38) on PACAP-induced changes in O_2 consumption, CO_2 production and energy expenditure. The doses of PACAP and PACAP (6–38) were 188 and 940 pmol, respectively. The number of chicks in each group was as follows: saline, 6; PACAP alone, 5; and PACAP plus PACAP (6–38), 6. In the saline and PACAP plus PACAP (6–38) groups, 7 ($n=3$, at 36, 39, 42, 48, 54 and 57 min) and 1 ($n=1$, at 36 min) samples respectively could not be measured for O_2 consumption owing to technical problems. Data are expressed as means±S.E.M.

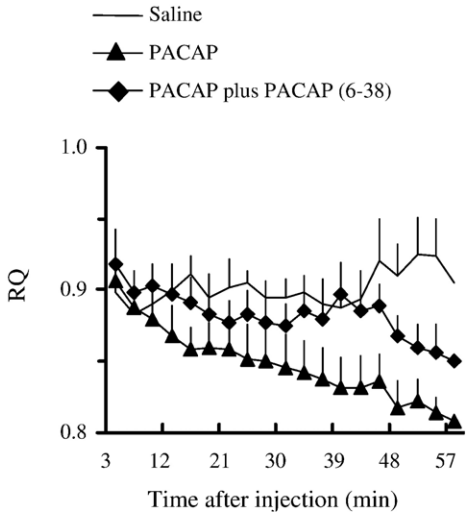

Fig. 7. Effect of ICV co-injection of PACAP (6–38) on PACAP-induced changes in RQ. The doses of PACAP and PACAP (6–38) were 188 and 940 pmol, respectively. The number of chicks in each group was as follows: saline, 6; PACAP alone, 5; and PACAP plus PACAP (6–38), 6. In saline and PACAP plus PACAP (6–38) groups, 7 ($n=3$, at 36, 39, 42, 48, 54 and 57 min) and 1 ($n=1$, at 36 min) samples respectively could not be measured for O_2 consumption owing to technical problems. Data are expressed as means±S.E.M.

locomotor activity was not altered by VIP and PACAP in the present study, indicating that VIP and PACAP might affect energy metabolism directly in chicks. However, it should be noted that since Masuo et al. (1995) used higher doses of PACAP (1 and 2 nmol), it is possible that PACAP-induced locomotor activity might be dose-dependent.

The idea that VIP and PACAP affect energy metabolism directly is also supported by the finding in the present study that ICV injection of these both peptides decreased RQ (Fig. 3). The decreased RQ suggests that these peptides act in the chick brain to induce lipid utilization. The present study also showed that ICV injection of VIP or PACAP increased plasma NEFA concentrations, together with a tendency to decrease plasma TG concentrations (Fig. 4). These results are indicative of the effects of VIP and PACAP in increasing lipolysis, resulting in increased plasma NEFA concentrations. In the case of VIP, the hypothesis that this peptide induces lipid utilization is strengthened by the fact that ICV injection of VIP also decreased plasma GLU concentration (Fig. 4). The effects of VIP and PACAP on plasma lipid metabolism seem to be similar. However, the PACAP-induced decrease in RQ was partly attenuated by PACAP (6–38) (Fig. 7), demonstrating that there may be PACAP-specific mechanisms involved in the regulation of lipid metabolism in chicks. The effects of the peptides on GLU metabolism also differed. Thus, ICV injection of VIP decreased plasma GLU concentration while PACAP did not (Fig. 4). In addition, ICV injection of VIP decreased RQ to about 0.7 at the end of experiment (Fig. 3) while RQ reached to about 0.8 by the PACAP treatment (Figs. 3 and 7). From these lines of evidences, PACAP-induced change in energy source would be different from the case of VIP. More detailed clarification will be done in the future.

In the present study, we used only one dose (940 pmol) of PACAP (6–38) to antagonize the effect of PACAP. The dose of PACAP (6–38) did not fully attenuate the PACAP-induced changes in rectal temperature and RQ. It is therefore possible that the dose used was not sufficient to attenuate the effects of PACAP completely. Alternatively, there might be PAC1 receptor-independent pathways involved in the mediation of PACAP's effects, for example, VPAC receptor pathway. These issues remain to be addressed in future studies.

In summary, the present study suggested that the brain VIP and PACAP are regulators of energy metabolism in the chick but that their mechanisms of action are different.

Acknowledgements

This work was supported by Grant-in-Aid for Young Scientists (Nos. 15780187 and 18780203), Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science (Nos. 16380191, 17208023 and 18208023) and Inamori Foundation.

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