# グルカゴン様ペプチド-1(GLP-1)の脳室投与は孵化直後ニワトリヒナの飲水量を低下させる


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# Intracerebroventricular Injection of Glucagon-Like Peptide-1 Inhibits Water Intake of the Neonatal Chick

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The aim of present study was to elucidate whether central injection of GLP-1 modifies water intake of chicks under dehydration or angiotensin II (AII) treatment. Birds (2-day-old) with or without 19-h dehydration were given diets and then GLP-1 (30 ng/10$\mu l$) or saline was injected to birds in both treatments by the intracerebroventricular (i.c.v.) route in experiment 1. Irrespective of drinking conditions GLP-1 inhibited water consumption for 1 h ; the inhibitory effect of water intake by GLP-1 was strong in the dehydrated birds. Central administration of AII (0, 25, 50 and 100 ng) increased water intake for 30 min in a dose dependent manner when water given *ad libitum* in experiment 2. Thereafter, the relationships between i.c.v. GLP-1 and AII on water intake was investigated in experiment 3. Water intake was decreased by co-injection with GLP-1 (30 ng) and AII (100 ng).

These results suggest that central GLP-1 may be the regulator of water intake in the neonatal chick.

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**Key words** : glucagon-like peptide-1 (GLP-1), angiotensin II, dehydration, water intake, central nervous system

## Introduction

In the chicken, there are several reports that angiotensin II (AII) induces water intake (SNAPIR *et al.*, 1976 ; FIRMAN and VOLMERT, 1991 ; VOLMERT and FIRMAN, 1992). AII induces drinking by acting on agiotensin receptors in the brain. Recently, NAVARRO *et al.* (1996) and TANG-CHRISTENSEN *et al.* (1996) reported that glucagon-like peptide-1 (GLP-1) inhibited food intake and also water intake in the rat. However, little is known about other peptides related to water intake in the chicken.

In the present study, in order to understand further the water intake system in neonatal chicks, we have examined the potency of GLP-1 for water intake under the conditions in which drinking is stimulated by dehydration or AII.

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## Materials and Methods

Day-old broiler chicks of both sexes were purchased from a local hatchery (Fusoen, Aichi, Japan). Birds were maintained in a room with 24 h light and at a temperature of 28℃. They were given free access to a commercial starter diet (Nihon Nosan Kogyo Co. Ltd., Tokyo, Japan). Birds were distributed into experimental groups based on their body weight, so that average body weight was as uniform as possible within the same experiment. Chicken GLP-1 (7-36) amide and human AII (octapeptides) were purchased from Peptide Institute, Inc. (Osaka, Japan) and Cosmo Bio Co., Ltd. (Tokyo, Japan), respectively. We used chicken GLP-1 (7-36) amide in the present study, because GLP-1 (7-36) amide has been reported to be the most abundant form secreted from the L-cell (FEHMANN *et al.*, 1995). Peptide was dissolved in a 0.1% Evans Blue solution, which was prepared in 0.85% saline.

Treated (19-h dehydration) and freely drunk birds were fed *ad libitum* in experiment 1. Birds (2-day-old, 9 birds per group) in both treatments were given water, but not food, for 1 h after i. c. v. administration of GLP-1 (30 ng) or saline with the solutions (10 $\mu l$) using a microsyringe according to the methods by DAVIS *et al.* (1979).

In experiment 2, birds (2-day-old, 8 birds per group) having free access to food and water were injected by the i.c.v. route with four levels (0, 25, 50 and 100 ng) of AII and then were freely given water alone for 30 min.

In experiment 3, birds (2-day-old, 9 birds per group) were reared under conditions similar to experiment 2 and were administered i.c.v. with saline, GLP-1 (30 ng), AII (100 ng) or both GLP-1 and AII. Birds were freely given water alone for 30 min.

At the end of the experiments, birds were killed by decapitation, followed by brain sectioning to identify location of drug injection. Figure 1 shows the distribution of Evans Blue dye in the lateral ventricle. Data from the individuals that were not verified by the presence of Evans Blue dye in the lateral ventricle were discarded. The number of birds used was : *ad libitum* drinking with i.c.v. saline, 9 ; *ad libitum* drinking with i.c.v. GLP-1, 8 ; dehydration with i.c.v. saline, 9 ; dehydration with i.c.v. GLP-1, 8

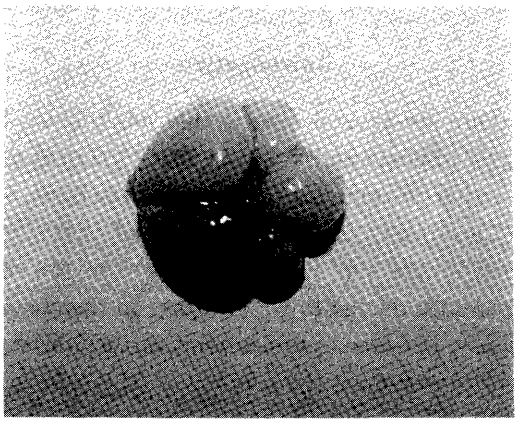

Fig. 1. The distribution of Evans Blue dye in the lateral ventricle of the neonatal chick.

in experiment 1 : saline, 7 ; AII 25 ng, 7 ; AII 50 ng, 7 ; AII 100 ng, 7 in experiment 2 : saline, 8 ; AII, 8 ; GLP-1, 8 ; AII with GLP-1, 9 in experiment 3.

Data were subjected to two-way (experiments 1 and 3) and one-way (experiment 2) analysis of variance by the General Linear Model procedure using a commercially available package (SAS, 1985), and comparisons between means were made using Duncan's multiple range test. The results were indicated as the mean±S.E.M.

## Results

Figure 2 shows the water intake of dehydrated or control birds injected i.c.v. with GLP-1 or saline in experiment 1. Water intake was significantly ($F$ (30, 1)=28.44, P< 0.001) inhibited by injection of GLP-1 and significantly ($F$ (30, 1)=414.59, P<0.001) enhanced by dehydration. A significant interaction ($F$ (30, 1)=14.70, P<0.001) was observed between dehydration and GLP-1, implying that the inhibitory effect of GLP-1 on water consumption would be more readily induced in birds when drinking behavior is stimulated..

As shown in Fig. 3, small amounts of central AII efficiently enhanced water intake in a dose dependent manner ($F$ (24, 3)=4.65, $P$<0.05).

Figure 4 demonstrates water consumption of chicks treated with i.c.v. GLP-1, AII, or both. Central GLP-1 significantly ($F$ (29, 1)=5.87, $P$<0.05) suppressed water intake and the reverse was true for central AII ($F$ (29, 1)=49.19, $P$<0.001). No significant ($F$ (29, 1)=2.17, $P$>0.05) interaction was detected between GLP-1 and AII.

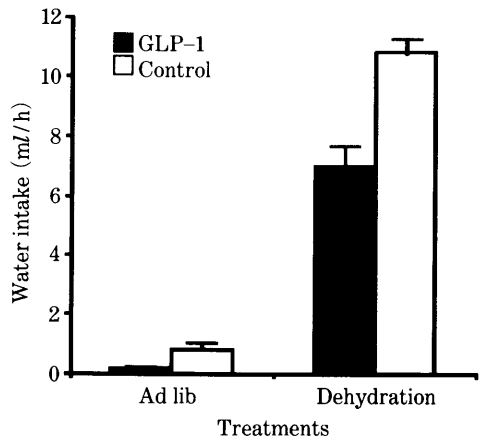


Fig. 2. Water intake (over 1 h) of chicks administered i.c.v. with chicken glucagon-like peptide-1 (GLP-1 ; 30 ng) or saline in dehydrated (19 h) or control birds. Birds were given water *ad libitum*, but not food. Values are means±S.E.M. The number of birds used was : *ad libitum* drinking with i.c.v. saline, 9 ; *ad libitum* drinking with i.c.v. GLP-1, 8 ; dehydration with i.c.v. saline, 9 ; dehydration with i.c.v. GLP-1, 8. Significance levels : GLP-1, $P$<0.001 ; dehydration, $P$<0.001 and interaction between GLP-1 and dehydration, $P$<0.001.

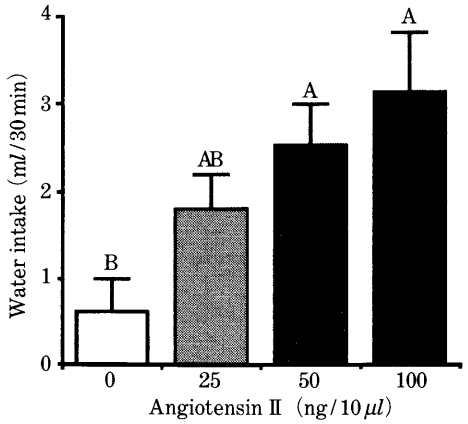


Fig. 3. Water intake (over 30 min) of chicks injected i.c.v. with graded levels of angiotensin II (0, 25, 50 or 100 ng). Birds were given water *ad libitum*, but not food. Values are means±S.E.M. The number of birds used was : saline, 7 ; AII 25 ng, 7 ; AII 50 ng, 7 ; AII 100 ng, 7. Means with a different letter are significantly different at $P<0.05$.

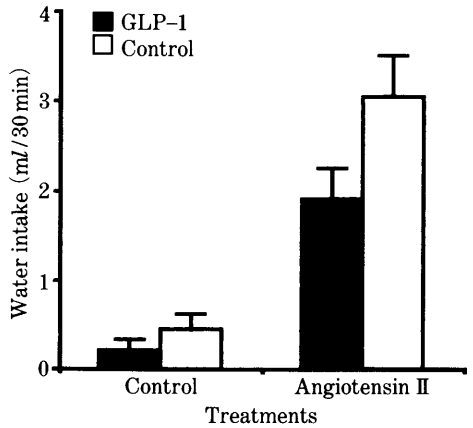


Fig. 4. Water intake (over 30 min) of chicks injected i.c.v. with angiotensin II (AII ; 100 ng), glucagon-like peptide-1 (GLP-1 ; 30 ng) or both. Birds were given water *ad libitum*, but not food. Values are means±S.E.M. The number of birds used was : saline, 8 ; AII, 8 ; GLP-1, 8 ; AII with GLP-1, 9.
Significance levels : GLP-1, $P<0.05$ ; AII, $P<0.001$ and interaction between GLP-1 and AII, $P>0.05$.

## Discussion

Water consumption induced by dehydration was inhibited by central GLP-1 in the first experiment. It is well known that plasma concentration of AII increases during dehydration (Willis, 1998). Thus, the following experiments were done to elucidate

the relationship between central GLP-1 and AII. In chickens, so far, it is confirmed that AII stimulates drinking behavior when this hormone is administered by routes such as intravenous, intracranial and subcutaneous injection (SNAPIR *et al.*, 1976 ; FIRMAN and VOLMERT, 1991 ; VOLMERT and FIRMAN, 1992). To authors' knowledge, however, effective doses of i.c.v. AII on drinking have not been investigated in the neonatal chick. Accordingly, we firstly studied the central effect of AII on drinking of the neonatal chick. Small amounts of central AII clearly enhanced water intake in a dose dependent manner. Consequently, the relationship between AII and GLP-1 on drinking was investigated by using the highest dose (100 ng) of AII and a dose of 30 ng of GLP-1 in which this level mildly inhibited food intake of the neonatal chick (FURUSE *et al.*, 1997a,b). Central GLP-1 suppressed water intake enhanced by central AII. In rats, TURTON *et al.* (1996) demonstrated that central GLP-1 depressed food intake. In the subsequent report by NAVARRO *et al.* (1996) and TANG-CHRISTENSEN *et al.* (1996), not only food intake but water consumption induced by AII was decreased by central administration of GLP-1 in the rat. Taken together with the previous reports (FURUSE *et al.*, 1997a,b), in the neonatal chick, it is concluded that central GLP-1 may be one of the important peptides involved in the regulation of both feeding and drinking behavior. However, the mechanism by which GLP-1 suppressed food and water intake may be different for different animals. For instance, TANG-CHRISTENSEN *et al.* (1996) reported that after i.c.v. injection the significant difference in motility between GLP-1 treated, saline-treated, or uncannulated control animals did not occur in the rat. On the other hand, we observed that the frequency of pecking behavior was significantly reduced after central administration of GLP-1 in the neonatal chick (Bungo *et al.*, 1999). Furthermore, LARSEN *et al.* (1997) reported that i.c.v. injection of GLP-1 increased plasma corticosterone and suggested that the relationship between suppressed food intake and the activities of the hypothalamic-pituitary-adrenal axis of rats. On the contrary, plasma corticosterone concentration was not changed after i.c.v. injection of GLP-1 in the neonatal chick (FURUSE *et al.*, 1997 c). The central mechanism of the action of GLP-1 on food and water intake remains to be studied in the neonatal chick.

The results presented here suggest that central GLP-1 may interact with AII and may be one of the inhibitor of water intake in the chicken.

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# グルカゴン様ペプチド-1（GLP-1）の脳室投与は孵化直後ニワトリヒナの飲水量を低下させる

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本研究は，脱水下あるいはアンギオテンシンII（AII）投与下のヒナの飲水量を中枢におけるGLP-1が修飾するか否かを明らかにすることを目的として行った。ヒナ（2日齢）には自由摂食条件下で自由飲水または19時間の脱水処理を施し，その後，両飲水処理したヒナの脳室に生理的食塩水またはGLP-1（30 ng/10 $\mu l$）を投与した（実験1）。飲水処理の有無に関わらずGLP-1はヒナの1時間当たりの飲水量を減少させたが，その効果は脱水処理で著しかった。AII（0, 25, 50 and 100 ng）をヒナの中枢に投与すると，用量依存的に投与後30分間の飲水量が増加した（実験2）。その後，中枢投与したGLP-1とAIIの交互作用を調査した（実験3）。GLP-1（30 ng）とAII（100 ng）の同時投与により飲水量は減少した。

これらの結果から，孵化直後のヒナにおいては中枢におけるGLP-1が飲水量の調節因子の一つである可能性が示唆された。

（家禽会誌 ***36*** *: 229-235, 1999*）

**キーワード**：グルカゴン様ペプチド-1（GLP-1），アンギオテンシンII（AII），脱水，飲水，中枢神経系