

# INFLUENCE OF SEROTONERGIC TRANSMISSION AND POSTSYNAPTIC 5-HT<sub>2C</sub> ACTION ON THE FEEDING BEHAVIOR OF *Coturnix japonica* (GALLIFORMES: AVES)

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## ABSTRACT

We investigated the role of 5-HT<sub>2C</sub> receptors and serotonergic transmission in the feeding behavior control of quails. Administration of serotonin releaser, fenfluramine (FEN) and 5-HT<sub>2C</sub> agonists, mCPP and MK212, 1.0 and 3.3 mg/Kg induced significant inhibition of food intake in previously fasted fowls (0.71 ± 0.18 g and 0.47 ± 0.2 g; 0.49 ± 0.22 g and 0.48 ± 0.29 g; 0.82 ± 0.13 g and 0.71 ± 0.16 g, respectively). Control groups ranged from 2.89 ± 0.21 g to 2.97 ± 0.22 g, 60 min after reintroduction of food, P < 0.0001). Similar results were obtained with normally fed quails. Both serotonin releaser and 5-HT<sub>2C</sub> agonists, in a 3.3 mg/Kg dose, induced hypophagy (FEN, 0.78 ± 0.08 g; mCPP, 0.89 ± 0.07 g; MK212, 1.25 ± 0.17 g vs. controls, 2.05 ± 0.12 g, 120 min after food was presented, P < 0.0001 to P < 0.01). Previous administration of 5-HT<sub>2C</sub> antagonist, LY53857 (5.0 mg/Kg) blocked the hypophagic response induced by 5-HT<sub>2C</sub> agonists 60 min after food was reintroduced. Current data show a modulatory role of serotonin release and postsynaptic 5-HT<sub>2C</sub> receptors in the feeding behavior of quails.

**Keywords:** serotonergic transmission, serotonin release, 5-HT<sub>2C</sub> receptors, food intake, quails, *Coturnix japonica*.

## RESUMO

### **Influência da transmissão serotoninérgica e da ação 5-HT<sub>2C</sub> pós-sináptica no comportamento alimentar em *Coturnix japonica* (Galliformes: Aves)**

Investigamos nesse estudo o papel dos receptores 5-HT<sub>2C</sub> e da transmissão serotoninérgica no controle do comportamento alimentar em codornas. Em grupo de aves em jejum, a administração do liberador de serotonina, fenfluramina (FEN) e dos agonistas 5-HT<sub>2C</sub>, mCPP e MK212, nas doses de 1,0 e 3,3 mg/Kg induziu a uma redução significativa da ingestão alimentar (0,71 ± 0,18 g e 0,47 ± 0,2 g; 0,49 ± 0,22 g e 0,48 ± 0,29 g; 0,82 ± 0,13 g e 0,71 ± 0,16 g; respectivamente). A ingestão de alimento nos grupos controles variou de 2,89 ± 0,21 g a 2,97 ± 0,22 g, 60 min após a reapresentação de alimento, P < 0,0001). Resultados similares foram obtidos com as codornas normoalimentadas. Tanto o liberador de serotonina, FEN, quanto os agonistas 5-HT<sub>2C</sub>, mCPP e MK212 em doses de 3,3 mg/Kg induziram resposta hipofágica (FEN, 0,78 ± 0,08 g; mCPP, 0,89 ± 0,07 g; MK212, 1,25 ± 0,17 g vs. controles, 2,05 ± 0,12 g, 120 min após a oferta de alimento, P < 0,0001 a P < 0,01). A administração prévia do antagonista 5-HT<sub>2C</sub>, LY53857 (5,0 mg/Kg) bloqueou a resposta hipofágica induzida pelos agonistas 5-HT<sub>2C</sub>, 60 min após a apresentação

de alimento. Os resultados obtidos demonstram o papel modulatório da liberação de serotonina e dos receptores pós-sinápticos 5-HT<sub>2C</sub>, no controle do comportamento alimentar de codornas.

*Palavras-chave:* transmissão serotoninérgica, liberação de serotonina, receptores 5-HT<sub>2C</sub>, ingestão de alimento, codornas, *Coturnix japonica*.

## INTRODUCTION

Previous studies involving mammals, particularly murines, implicated the brain serotonergic system in the control of appetite and satiety. Early investigations in this field showed that increased circulating tryptophan availability (initial precursor of serotonin synthesis), and the subsequent increasing brain serotonin content, constitute physiological parameters for activation of satiety and modulation of appetite (Fernstrom & Wurtman, 1971a, 1971b; Fernstrom, 1983; Tyce, 1990). Feeding response in rats is accompanied by an increase in serotonin release in the lateral hypothalamic area, a region involved with coordination of feeding behavior (Shimizu *et al.*, 1992).

The participation of 5-HT<sub>2C</sub> and 5-HT<sub>1B</sub> receptors is a widely accepted notion in the anorectic response in several paradigms (Blundell, 1984; Blundell & Hill, 1987; Curzon, 1990; Halford *et al.*, 1997). Involvement of serotonergic transmission demonstrated by treatment with serotonin releasers has also confirmed that system's modulatory influence on feeding behavior (Blundell, 1984). On the other hand, inhibition of the firing rate of raphe serotonergic neurons and the resulting decrease of ascending transmission elicit hyperphagy (Dourish *et al.*, 1986; Hutson *et al.*, 1986, 1988; Aghajanian *et al.*, 1990; Curzon, 1990, 1991).

A few reports have shown a physiological correlation between serotonergic system activity and control of feeding behavior in birds (Rosebrough, 1996; Steffens *et al.*, 1997; Thomaz *et al.*, 1998; Saadoun & Cabrera, 2002; Reis *et al.*, 2005).

Traits of serotonergic control of the feeding behavior are probably already present to a high degree in birds, since the topographical and functional organization of the serotonergic system preserves similarities with that of reptiles and mammals (Dubé & Parent, 1981; Parent, 1981; Parent *et al.*, 1981; Duchala *et al.*, 1984; Sako *et al.*, 1986; Cozzi *et al.*, 1991; Challet *et al.*, 1996; Gruss & Braun, 1997). Nevertheless, the biochemical,

pharmacological and behavioral characterization of serotonin receptors has been little investigated (Gleeson *et al.*, 1992; Saadoun & Cabrera, 2002).

Studies of the participation of 5-HT<sub>2C</sub> receptors and serotonin release in the control of feeding behavior have not been reported, although binding sites in bird brains for classical serotonergic agonists have been described (Stephenson & Andrew, 1994; Saadoun & Cabrera, 2002).

Observations in our laboratory revealed evidence of serotonergic involvement in the control of quail feeding behavior (Reis *et al.*, 2005). In this report, we demonstrate that dietary supplementation of tryptophan (initial precursor of serotonin synthesis) inhibits food intake, although a much larger concentration than that required to produce the same anorectic response in mammals is necessary.

When 5-hydroxy-tryptophan, the immediate precursor of serotonin synthesis, was administered by the systemic route, the response elicited was hypophagy associated with intense thirst. However, the role of synaptic transmission and the relevance of the postsynaptic action of serotonin releasers and serotonergic agonists on the feeding behavior of quails are still unknown.

Thus, this paper examines the influence of treatment with the serotonin releasers fenfluramine and 5-HT<sub>2C</sub> agonists mCPP and MK212 on the feeding behavior of fasted and normally fed quails.

## MATERIALS AND METHODS

### *Animals and materials used*

Adult male quails weighing 130-160 g were kept in special cages equipped with drinking fountains and individual food containers. Throughout the birds' adaptation period to the laboratory conditions they had *ad libitum* access to food and water (Purina, 20% raw protein for quails) and were exposed to a 12/12 h light/dark cycle and a room temperature of 25 to 30 °C. Food intake was evaluated with a precision electronic scale. The drugs used here, i.e., fenfluramine, releaser

of serotonin (FEN) (Sigma-Aldrich, USA) and mCPP [1-(3-chlorophenyl) piperazine] (Research Biochemical Inc., USA) and MK212 [6-chloro-2-(1-piperazinil) pyrazine] (Merck Sharp & Dohme, USA), 5-HT<sub>2C</sub> agonists were all administered by subcutaneous (sc) route 30 min before food was offered. The 5-HT<sub>2C</sub> antagonist LY53857 (Eli Lilly, USA) was administered 30 min, sc, before injection of the 5-HT<sub>2C</sub> agonists.

### ***Experimental procedures***

#### *Influence of the treatment with serotonin releaser FEN and the 5-HT<sub>2C</sub> agonists mCPP and MK212 on the food intake of fasted quails*

The birds were fasted from 7:00 to 13:00 h, at which time they were fed. Food intake was measured during 60 min. To evaluate the influence of serotonin release on the birds' feeding behavior, they were given doses of 1.0 (N = 10) and 3.3 mg/Kg (N = 10) of FEN. The influence of 5-HT<sub>2C</sub> receptors on the food intake behavior was evaluated by treating the birds with doses of 1.0 (N = 10) and 3.3 mg/Kg (N = 12) of mCPP and with doses of 1.0 (N = 10) and 3.3 mg/Kg (N = 12) of MK212. The control groups for all conditions were treated with isotonic saline (0.1 mL/100 g) (N = 10).

#### *Influence of the treatment with serotonin releaser FEN or 5-HT<sub>2C</sub> agonists mCPP and MK212 on the food intake of normally fed quails*

Food intake was also evaluated in a group of normally fed birds treated with a dose of 3.3 mg/Kg (N = 10 for each group) of FEN, mCPP and MK212 for 120 min. A control group of normally fed quails was treated with saline (0.1 mL/100 g) (N = 11).

#### *Influence of previous treatment with 5-HT<sub>2C</sub> antagonist LY53857 on the food intake of normally fed quails*

In this condition, 5-HT<sub>2C</sub> antagonist LY53857 (5.0 mg/Kg) was administered 30 min before mCPP (3.3 mg/Kg, N = 10) and MK212 (3.3 mg/Kg, N = 11) injections. Other groups were formed and received only saline (N = 11), mCPP, 3.3 mg/Kg (N = 10) or MK212, 3.3 mg/Kg (N = 10).

### ***Statistical analysis***

The results of these treatments are expressed as mean  $\pm$  standard error. The values obtained

were statistically analyzed by ANOVA and Student "t" test. Differences among mean values were considered significant when  $P < 0.05$ .

## **RESULTS**

Administration of FEN, mCPP and MK212 in doses of 1.0 and 3.3 mg/Kg induced a significant decrease of food intake by fasted quails ( $0.71 \pm 0.18$  g and  $0.47 \pm 0.2$  g;  $0.49 \pm 0.22$  g and  $0.48 \pm 0.29$  g;  $0.82 \pm 0.13$  g and  $0.71 \pm 0.16$  g, respectively). The controls groups ranged from  $2.89 \pm 0.21$  g to  $2.97 \pm 0.22$  g, 60 min after food was again offered,  $P < 0.0001$  (Figs. 1, 2 and 3).

Similar results were obtained with the normally fed quails. Both the serotonin releaser FEN and the 5-HT<sub>2C</sub> agonists mCPP and MK212, in doses of 3.3 mg/Kg, induced a hypophagic response (FEN,  $0.78 \pm 0.08$  g; mCPP,  $0.89 \pm 0.07$  g; MK212,  $1.25 \pm 0.17$  g vs. controls,  $2.05 \pm 0.12$  g, 120 min after food was presented,  $P < 0.01$  to  $P < 0.0001$ ) (Fig. 4).

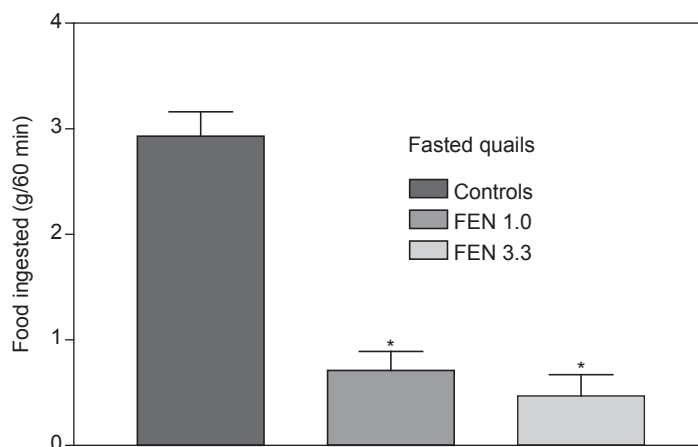
A previous injection of the 5-HT<sub>2C</sub> antagonist LY53857 (5.0 mg/Kg) blocked the hypophagic effect induced by mCPP and MK212, 60 min after food was offered. After 120 min, the blockage was maintained only with the mCPP treatment (Fig. 5).

## **DISCUSSION**

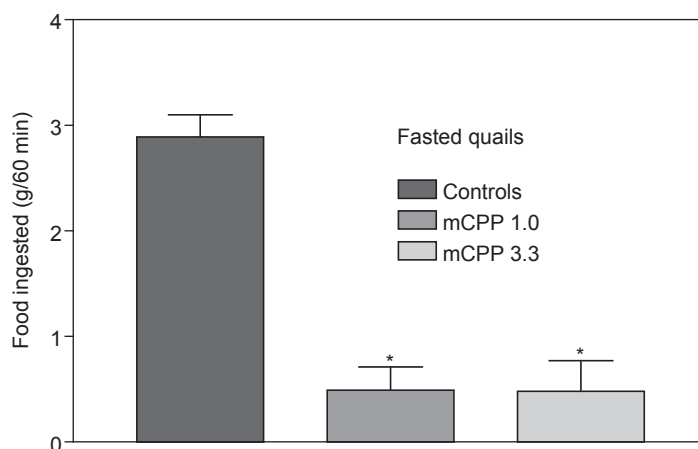
These results suggest the existence of serotonergic receptors in quails involved in the regulation of feeding behavior mediated by the activation of 5-HT<sub>2C</sub> receptors. In addition, the results attained with the serotonin releaser FEN indicate that serotonergic transmission is critically involved in the regulation of food intake by quails.

The agonists mCPP and MK212 also elicit an anorectic response in mammals by stimulation of the 5-HT<sub>2C</sub> receptor (Blundell, 1984; Blundell & Hill, 1987; Curzon, 1990; Halford *et al.*, 1997). These receptors are distributed in forebrain structures, particularly in the hypothalamus, which are known to be implicated in appetite control. Thus, the anorectic responses achieved with mCPP and MK212, which are powerful 5-HT<sub>2C</sub> agonists, provide evidence of the central mediation of appetite by serotonin.

The literature contains no report concerning the distribution and involvement of 5-HT<sub>2C</sub> receptors in the control of food intake by quails.



**Fig. 1** — Effect of the administration of the serotonin releaser, fenfluramine (FEN, 1.0 and 3.3 mg/Kg, sc), on the food intake of fasted quails. Data represent mean  $\pm$  SEM. \* $P < 0.0001$  compared with controls.



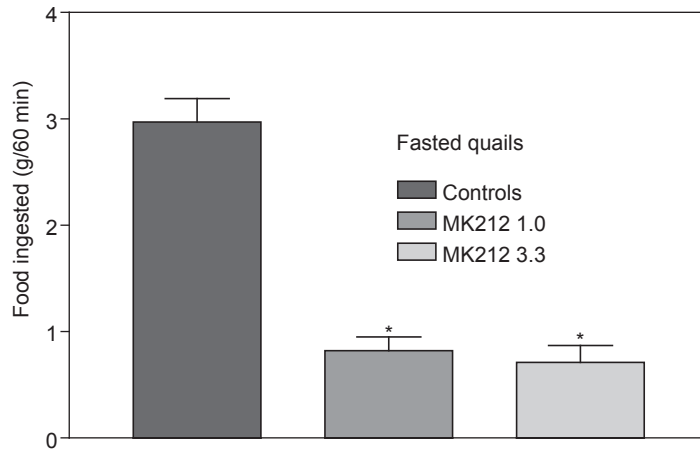
**Fig. 2** — Effect of the administration of 5-HT<sub>2C</sub> serotonin agonist, mCPP (1.0 and 3.3 mg/Kg, sc), on the food intake of fasted quails. Data represent mean  $\pm$  SEM. \* $P < 0.0001$  compared with controls.

Thus, our data represent the second report of indirect evidence of the involvement of a serotonergic system in the feeding behavior of quails. In the first report, Reis *et al.* (2005) demonstrated inhibition of food intake by quails treated with initial (tryptophan) and immediate (5-hydroxytryptophan) precursors of serotonin synthesis.

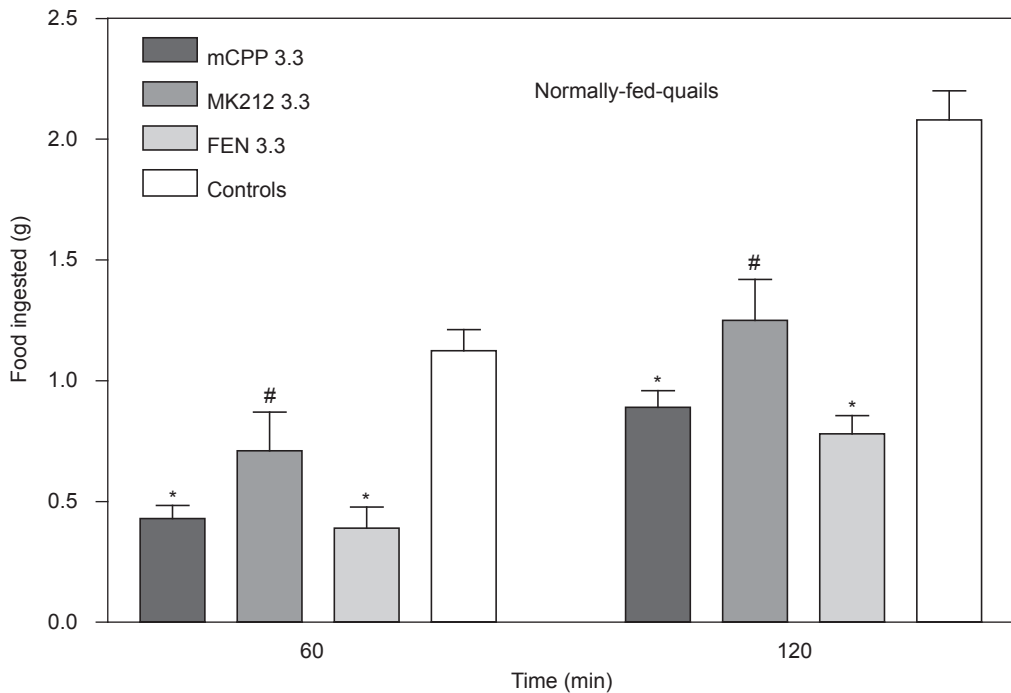
Current observations of the effects of FEN provide further evidence of the role of serotonergic transmission and serotonin release in the modulation of quail feeding behavior. Derivatives of that substance, which are used as anorexics for human obesity control, are also powerful anorexics

for other mammals (Blundell, 1984, 1991; Halford & Blundell, 2000). In this context, FEN has been widely used in humans to ascertain the release of brain serotonin in depressed patients with possible serotonergic hypofunction (Meltzer & Lowy, 1987).

Data on FEN suggest that the neurotransmission and release system of this indoleamine preserves molecular and functional characteristics found in mammals. The same observations apply to the results achieved with 5-HT<sub>2C</sub> agonists. Serotonin release and 5-HT<sub>2C</sub> postsynaptic activation in vertebrates probably constitute



**Fig. 3** — Effect of the administration of 5-HT<sub>2C</sub> serotonin agonist, MK212 (1.0 and 3.3 mg/Kg, sc), on the food intake of fasted quails. Data represent mean ± SEM. \*P < 0.0001 compared to controls.

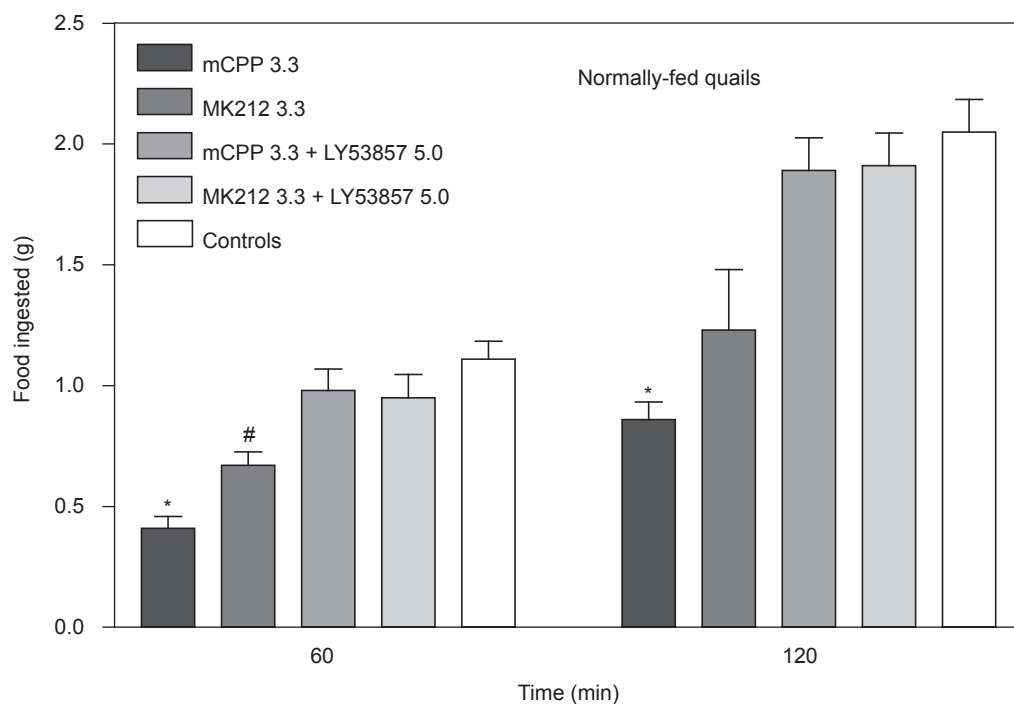


**Fig. 4** — Effect of the administration 5-HT<sub>2C</sub> serotonin agonists, mCPP and MK212 (1.0 and 3.3 mg/Kg, for each agonist, sc), and of serotonin releaser, fenfluramine (FEN, 1.0 and 3.3 mg/Kg, sc), on the food intake of normally fed quails. Data represent mean ± SEM. \*P < 0.0001 and #P < 0.01 compared with controls.

neurobiological substrata preserved since their evolutionary beginnings.

Along the evolutionary path, the preservation of molecules in these highly homologous systems possibly means that appetite modulation by

serotonin constitutes a homeostatic mechanism that has long been related with the control of energetic equilibrium. This reasoning is compatible with the assumption that topographic and functional phenotypes of the serotonergic system are



**Fig. 5** — Effect of previous administration of 5-HT<sub>2C</sub> antagonist, LY53857 (5.0 mg/Kg, sc), on the food intake of normally fed quails treated with 5-HT<sub>2C</sub> serotonin agonists mCPP and MK212 (1.0 and 3.3 mg/Kg for each agonist, sc). Data represent mean  $\pm$  SEM. \*P < 0.0001 and #P < 0.01 compared with controls.

phylogenically ancient and are implicated in the control of adaptive functions during increased afflux of metabolic/nutritional and viscerosensory signals to the brain.

Current data allow us to postulate that the functional characteristic of the neuronal system of serotonin transmission, namely serotonin release, and 5-HT<sub>2C</sub> postsynaptic activation, have been preserved from birds to mammals.

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