Involvement of neurotransmitters in the action of apelin-13 on passive avoidance learning in mice.

Gyula Telegdy [a,b], Agnes Adamik[b], Miklós Jászberényi[a]

Department of Pathophysiology[a], Neuroscience Research Group of the Hungarian Academy of Sciences[b], University of Szeged, Szeged, Hungary

Corresponding author: Gyula Telegdy
Dept. Pathophysiology
University of Szeged
Semmelweis 1
6721 Szeged, Hungary
Tel.: 36 62 545797
Fax: 36 62 545710

Abstract

The widespread distribution of apelin-13 and apelin receptors in the brain suggests an important function of this neuropeptide in the brain that has not been explored extensively so far. In the present work, apelin-13 was found facilitate the consolidation of passive avoidance learning in mice. In order to assess the possible involvement of transmitters in this action, the animals were pretreated with the following receptor blockers in doses which themselves did not influence the behavioral paradigm: phenoxybenzamine (a nonselective α-adrenergic receptor antagonist), propranolol (a β-adrenergic receptor antagonist), cyproheptadine (a nonselective 5-HT2 serotonergic receptor antagonist), atropine (a nonselective muscarinic acetylcholine receptor antagonist), haloperidol, (a D2, D3, D4 dopamine receptor antagonist), bicuculline (a γ-aminobutyric acid subunit **A** (GABA-A) receptor antagonist), naloxone (a nonselective opioid receptor antagonist), and nitro-L-arginine (a nitric oxide synthase inhibitor). Phenoxybenzamine, cyproheptadine, atropine, haloperidol, bicuculline and nitro-L-arginine prevented the action of apelin-13. Propranolol and naloxone were ineffective. The data suggest that apelin-13 elicits its action on the consolidation of passive avoidance learning via α-adrenergic, 5-HT2 serotonergic, cholinergic, dopaminergic, GABA-A-ergic and nitric oxide mediations.

Key words: apelin-13, passive avoidance learning, transmitter mediation.

1. Introduction

The first known apelin, apelin-36, isolated from bovine stomach extracts [23], is the endogenous ligand of an orphan G protein-coupled receptor, APJ, identified in a human gene by O'Dowd et al. (1993)[15]. It is derived from a 77-amino-acid precursor, preproapelin, identified in human and bovine tissue [23], which is processed to the molecular forms: apelin-36, apelin-26, apelin 19, apelin-17, apelin-13, apelin-12 in different tissues [9,17].

Synthetic C-terminal fragments of preproapelin, consisting of 13 to 19 amino acids, were found to exhibit significantly higher activities at the receptors than that of apelin-36 [9,24]. The most extensively studied. apelin-13 has been shown to participate in the regulation of cardiovascular function [11,19], fluid homeostasis [11,22], and the pituitary-adrenal axis [8,22]. Apelin receptors and apelin are widely distributed in the central nervous system (CNS) [3,6,13,18.], suggesting that apelin may be of importance in the regulation of certain CNS functions. We earlier demonstrated that apelin-13 increases the open-field activity, the plasma corticosterone level and the core temperature in male rats [8]. At present no data are available on the action of apelin-13 on learning and memory function or on the possible involvement of neurotransmitters in such action. The aim of the present work was to elucidate

the action of apelin-13 administered i.c.v. on passive avoidance learning in mice. The possible involvement of neurotransmitters in the passive avoidance learning was studied by pretreating the animals with certain neurotransmitter blockers.

2. Methods and Materials

2.1. Animals

CFLP male mice weighing 25-28 g, were used. The animals were kept and handled during the experiments in accordance with the Regulation of the Albert Szent-Györgyi Medical University Ethical Committee for the Protection of Animals in Research. Five animals per cage were housed in a light- (lights on at 0600 h and off at 1800 h) and temperature-controlled room (23 ^{o}C) and had free access to food and water .

2.2. Surgery

The mice were anesthetized with sodium pentobarbital (Nembutal 35 mg/kg i.p.) and a cannula was introduced into the lateral cerebroventricle and fixed to the skull with dental cement and acrylic resin. The animals were allowed to recover for 5 days. The correct location of the cannula was checked by dissecting the brain following completion of the experiments. Only animals with the correct location of the cannula were used in the evaluation of the experiments. All experiments were performed in the morning period.

2.3. Materials

Different doses of apelin-13 (Bachem, Bubendorf, Switzerland) dissolved in saline, or saline alone (control animals), in a volume of 2 μl, were injected i.c.v. into conscious rats. Apelin-13 in a quantity of 10 μg per ampoule was lyophilized and stored at −20 °C. Immediately before the experiments apelin-13 was dissolved in sterile pyrogen-free 0.9% saline and administered i.c.v. in a volume of 2 μl via a cannula.

2.4. Treatments

For the administration of the apelin-13 (Bachem, Bubendorf, Switzerland) a stainless steel cannula with an external diameter of 0.7 mm was implanted stereotaxically into the right lateral brain ventricle. The peptides were injected icv via the

cannula in a volume of 2 μl. For the transmitter interaction the most effective dose of apelin-13 (2 μg) was selected.

The antagonists of neurotransmitters was given intraperitoneally the Nitro-L-arginine was given icv. Following receptor blockers were used: Phenoxybenzamine hydrochloride was obtained from Smith Kline & French (Herts, UK); propranolol hydrochloride from ICI Ltd. (Macclesfield, UK); cyproheptadine hydrochloride from Tocris (Bristol, UK); atropine sulphate from EGYS (Budapest, Hungary); haloperidol from G. Richter (Budapest, Hungary); and bicuculline methiodide from Sandoz (Basle, Switzerland); naloxone hydrochloride (Endo Labs, Wilmington USA). Nitroω-L-Arginine methylester hydrochloride (Sigma St Louis USA).

2.5. Behavioural testing

2.5.1. Passive avoidance test

One-trial learning, step-through passive avoidance behavior was measured according to Ader et al. [2]. Briefly, mice were placed on an illuminated platform and allowed to enter a dark compartment. Since mice prefer dark to light, they normally entered within 5 s. Two additional trials were delivered on the following day. After the second trial, unavoidable mild electric footshocks (0.75 mA, 2 s) were delivered through the grid floor. Having entered the box, the animals could not escape the footshock. After this single trial, the mice were immediately removed from the apparatus and were treated. The consolidation of passive avoidance behavior was tested 24 h later. For consolidation, the animals were treated icv with Apelin-13, 1.0 and 2.0 ug. For further test the 2 μg was selected. The receptor blocker was given following the learning trial and 3o min later, the Apelin-13 (2 μg). In the 24 h testing each

animal was placed on the platform and the latency to enter the dark compartment was measured up to a maximum of 300 s.

2.6. Statistical analysis

The analysis of variance (ANOVA) test was followed by Tukey's test for multiple comparisons with unequal cell size. Probability values (*P*) of less than 0.05 are considered significant.

3. Results

Apelin-13 in a dose of 1 μg/2 μl i.c.v. had no action on the consolidation of passive avoidance learning, while 2 μg i.c.v. significantly facilitated the consolidation of passive avoidance memory [$F(2,30)=16.70$]; $p<0.05$ (Fig. 1).

In the phenoxybenzamine-pretreated group, apelin-13 (2 μg/2 μl i.c.v.) facilitated the consolidation of passive avoidance learning [$F(3,18)=12.70$]; $p<0.05$. Phenoxybenzamine (2 mg/kg i.p.) itself had no action, but fully blocked the action of apelin-13 (Fig. 2).

In the cyproheptadine-pretreated group, apelin-13 (2 μg/2 μl i.c.v.) facilitated the consolidation of passive avoidance learning [$F(3,18)=10.55$]; $p<0.05$. Cyproheptadine (l mg/kg i.p.) itself had no action, but fully blocked the action of apelin-13 (Fig. 3).

In the atropine-pretreated group, apelin-13 (2 μg/2 μl i.c.v.) facilitated the consolidation of passive avoidance learning [$F(3,19)=8.96$];$p<0.05$. Atropine (2 mg/kg i.p.) itself had no action, but fully blocked the action of apelin-13 (Fig. 4).

In the haloperidol-pretreated group, apelin-13 (2 μg/2 μl i.c.v.) facilitated the consolidation of passive avoidance learning [$F(3,19)=5.12$];$p<0,05$. Haloperidol (10 μg/kg i.p.) itself had no action, but fully blocked the action of apelin-13 (Fig. 5).

In the bicuculline-pretreated group, apelin-13 (2 μg/2 μl i.c.v.) facilitated the consolidation of passive avoidance

learning. [F(3,19)=12.76] ; p<0.05. Bicuculline (1 mg/kg i.p.) itself had no action, but fully blocked the action of apelin-13 (Fig. 6).

In the nitro-L-arginine- pretreated group, apelin-13 (2 µg/2 µl i.c.v.) facilitated the consolidation of passive avoidance learning [F(3,18=9,21)] ; p<0.05. Nitro-L-arginine (10 µg/2 µl i.c.v) itself had no action, but blocked the action of apelin-13 (Fig.7).

The propranolol and naloxone-pretreatment had no action on apelin-13-induced facilitation of the consolidation of passive avoidance learning (data are not shown).

4. Discussion

The wide-ranging distribution of apelin and apelin fibers both in the CNS and in the periphery suggested the important functions of apelin in physiological processes [3,6,13,18].

Apelin-13 has been found to increase the water intake [11,22] and to decrease the blood pressure [11,19], the latter in a nitric oxide-dependent manner [24]. Additionally it either increases [30], or reduces the food intake, depending on the mode of application of apelin-13 [20]. Its enhancement of the adrenal function [8,22], involves the participation of CRH [8,12] and vasopressin [12].

Apelin-13 causes a body temperature elevation [8,30], which seems to be prostaglandin-dependent [8]. Apelin-13 increases locomotion [30] and rearing in an open-field. Haloperidol, a dopamine receptor blocker, diminishes the action of apelin-13 [8]. Less is known about the direct action of apelin-13 on the central nervous system.

Apelin has been shown to exert neuroprotective action in a number of experimental conditions [5], in cultures it

markedly prevents apoptosis in mouse cortical neurons [31] and it protects hippocampal neurons against NMDA excitotoxity [14].

No data are available as yet regarding the action on learning and memory functions. The present work demonstrates that apelin-13 administered i.c.v. improves learning and memory consolidation in passive avoidance learning. Since learning and memory function are rather complicated processes, involving not only learning, but intention, fear, emotion, motivation, locomotion, etc. it is not surprising that a number of transmitter mediations are involved in these processes. A cholinergic mechanism seems to be one of the important means of transmission of the action of certain neuropeptides in memory consolidation processes. Our earlier work demonstrated that practically all of the neuropeptides which improved the memory in a passive avoidance paradigm, involved this form of transmission.(e.g. Telegdy, G. 1984. [25]. Alpha-adrenergic, serotonergic, dopaminergic and GABA-A-ergic mechanisms seems to be important. It would be interesting to establish, which transmitters are involved in certain components of a learning processes, which will lead to a memory consolidation. It seems that nitric oxide is involved not only in the learning process [27] but also in the blood pressure-lowering action of apelin-13 [24].

Naloxone and propranolol were ineffective in the doses used in these experiments, however the same dose of naloxone could block the action of growth hormone-releasing hormone antagonist in a similar test [28], furthermore propranolol attenuated the action orexin A and urocortin in the passive avoidance test [26, 29] and fully blocked the pituitary adenylate cyclase activating polypeptide action in the open-field activity [1].

Most of the evidence suggests that apelin-13 is mainly a neuromodulator, acting via certain transmitters eliciting a given action.

Finally, in view of the multiple actions of apelin-13 in physiological and pathophysiological processes, which are not reviewed in detail in this paper, we fully agree with other authors [4,7,10,21] that apelin-13 and its analogues or antagonists could be targets for the development of drugs influencing pathological processes and are well worth further exploration.

5. Conclusion

The data presented demonstrate that apelin-13, administered i.c.v., facilitates the memory consolidation in a passive avoidance paradigm via number of transmitter mediation, acting as a neuromodulator.

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Highlight

Apelin-13 has been tested on passive avoidance learning following i.c.v administration in mice.

Apelin-13 facilitates the memory consolidation.

In the memory consolidation, the following transmitters are involved: alpha-adrenergic, serotonergic, cholinergic, dopaminergic, GABA-A-ergic and nitric oxide.

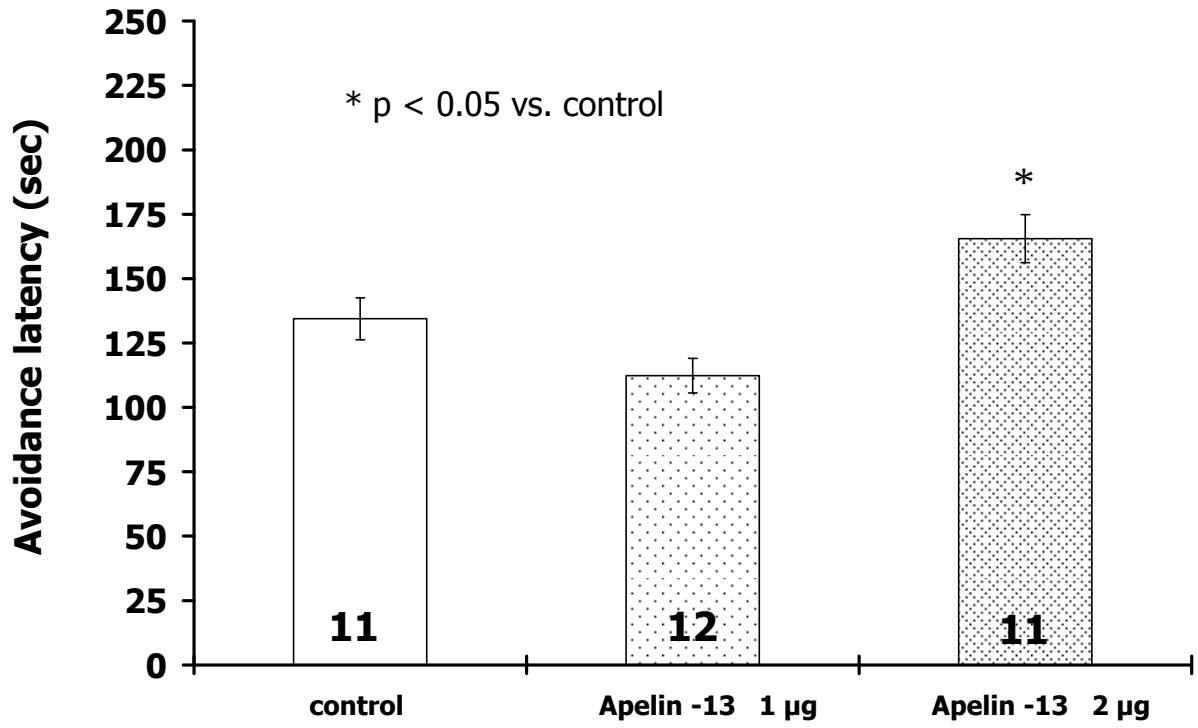

Fig.1

Fig.1.The action of different doses of apelin-13 administered i.c.v. immediatelly following the learning trial, on the consolidation of passive avoidance response. Data are expressed as means ± S.E.M. Number in bars are the numbers of animals used.

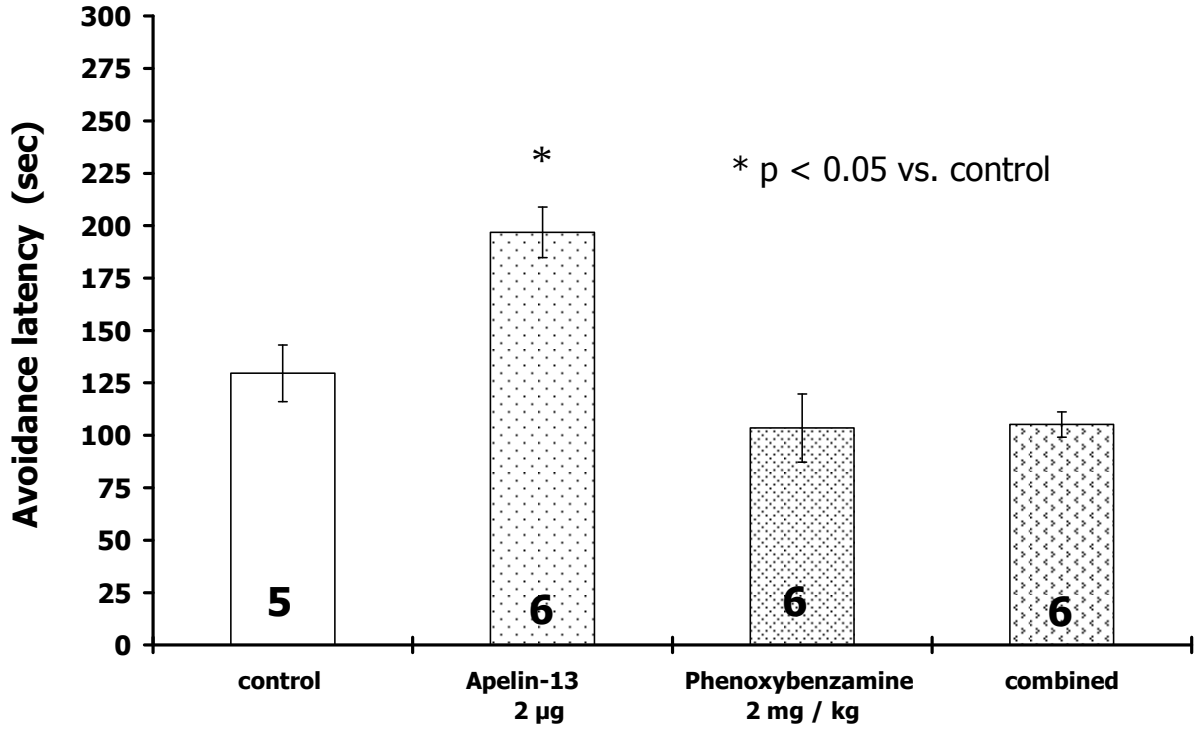

Fig.2.

Fig.2. The effect of a nonselective α–adrenergic receptor antagonist, phenoxybenzamine (2 mg/kg i.p) on apelin-induced (2 µg/2 µl i,c.v) consolidation of passive avoidance learning. . Data are expressed as means ± S.E.M. Number in bars are the numbers of animals used.

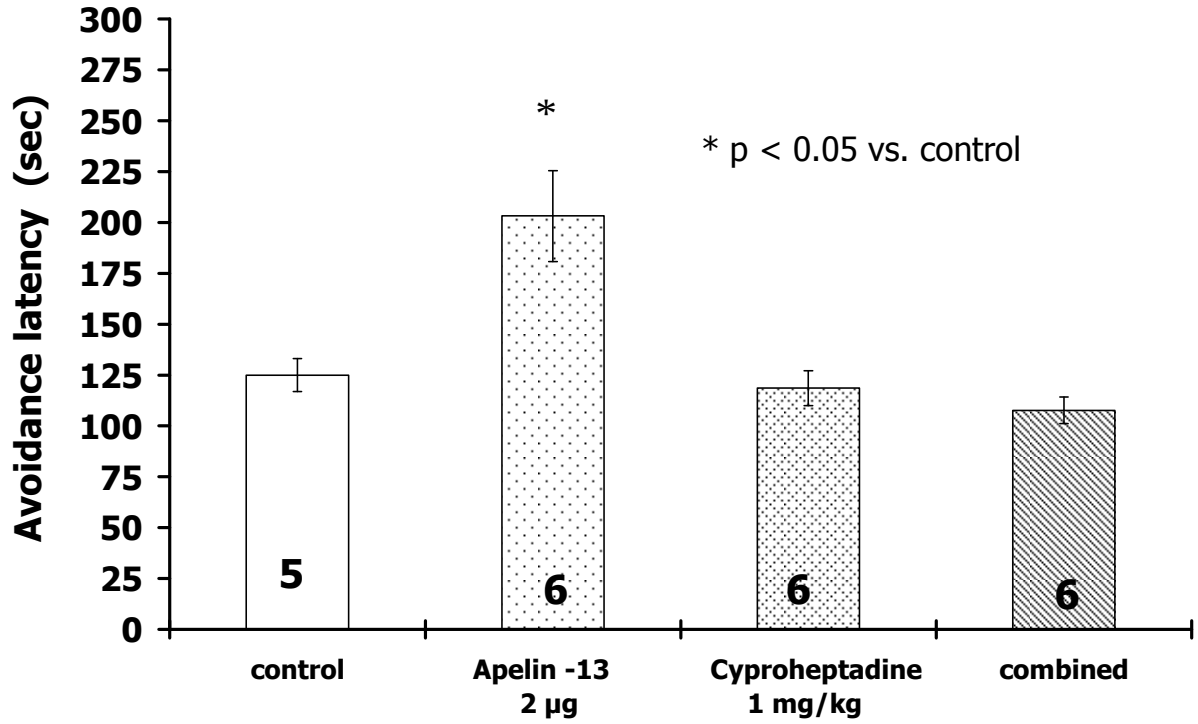

Fig.3.

Fig. 3. The effect of a nonselective 5-HT2 receptor antagonist, cyproheptadine (1 mg/kg i.p) on apelin-induced (2 µg/2 µl i.c.v) consolidation of passive avoidance learning. . Data are expressed as means ± S.E.M. Number in bars are the numbers of animals used.

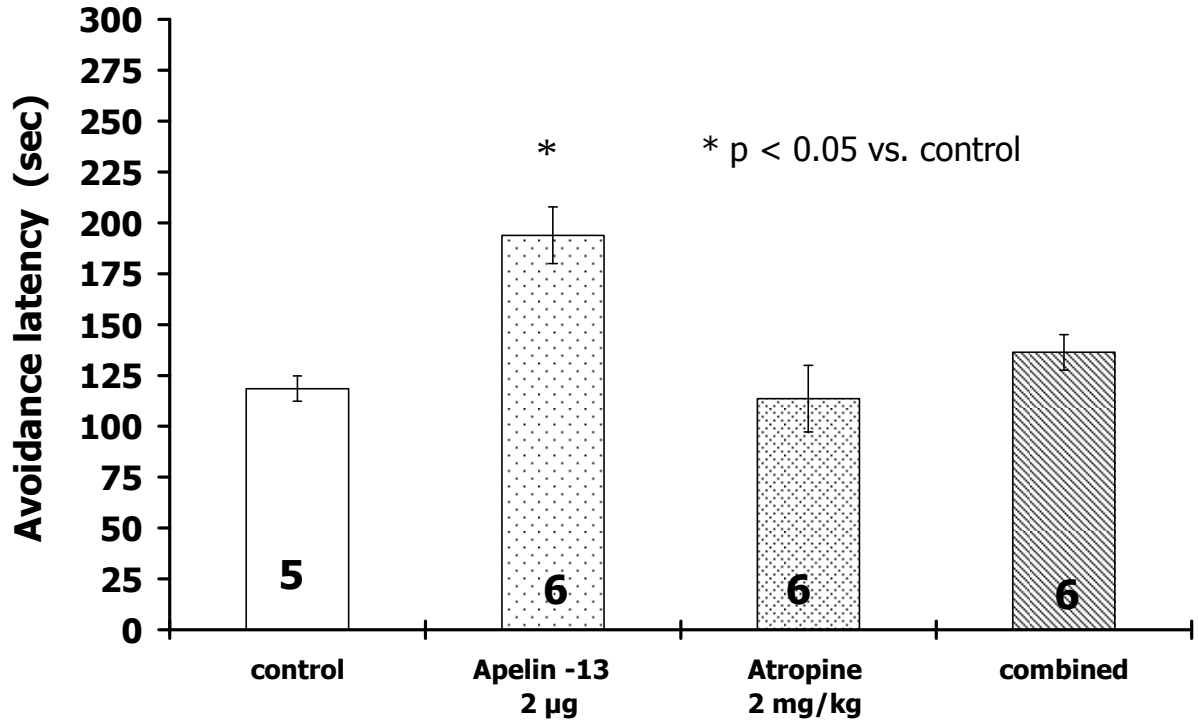

Fig. 4.

Fig.4. The effect of a nonselective muscarinic acethylcholine receptor antagonist, atropine (2 mg/kg i.p) on apelin-induced (2 µg/2 µl i.c.v) consolidation of passive avoidance learning. . Data are expressed as means ± S.E.M. Number in bars are the numbers of animals used.

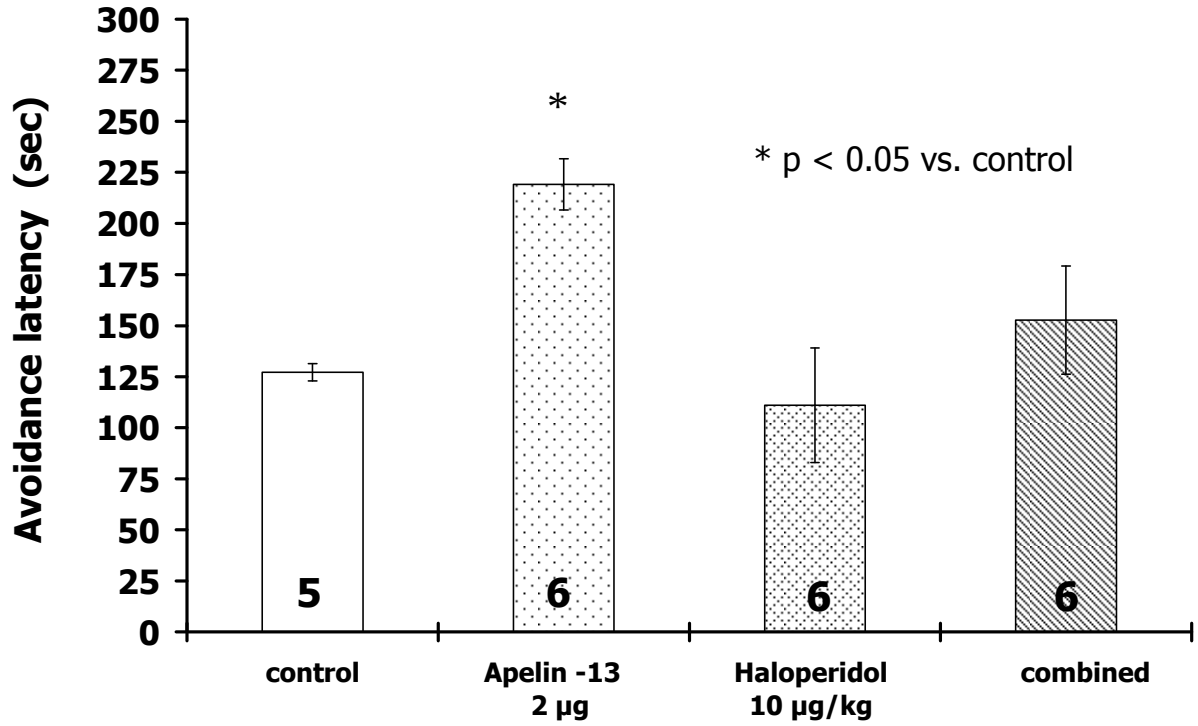

Fig.5.

Fig.5 . The effect of a D2,D3,D4 dopamine receptor antagonist, haloperidol 10 µg/kg i.p) on apelin-induced (2 µg/2 µl i.c.v) consolidation of passive avoidance learning. . Data are expressed as means ± S.E.M. Number in bars are the numbers of animals used.

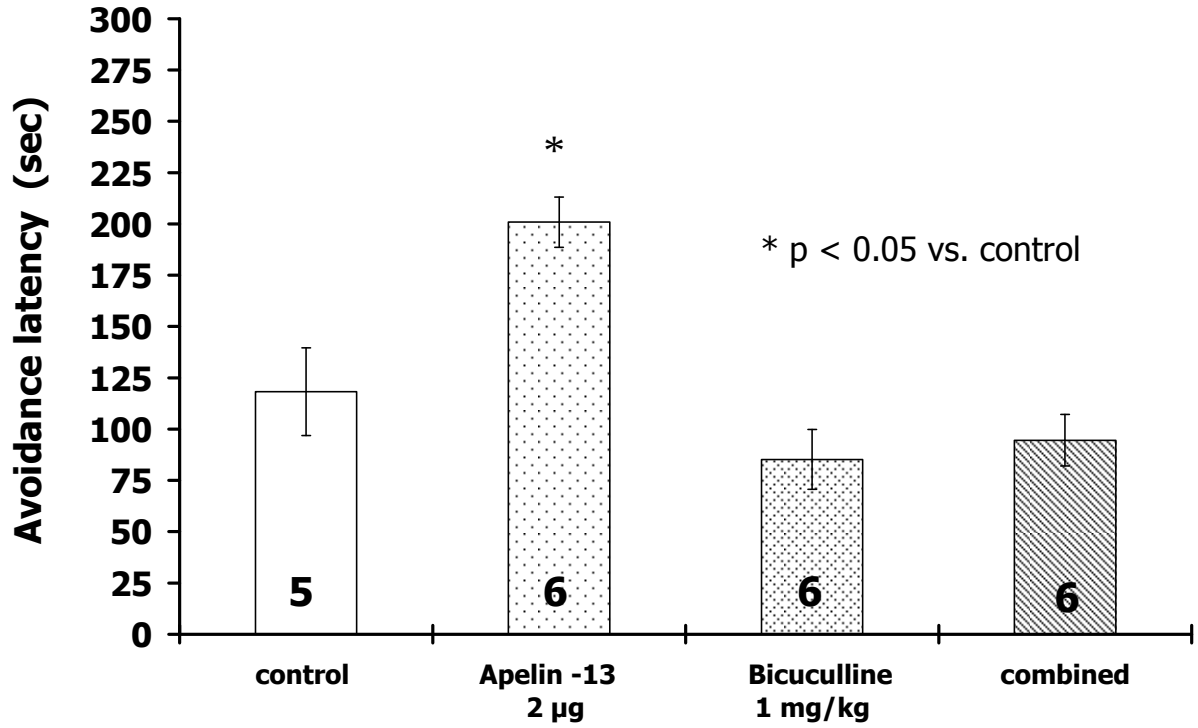

Fig.6.

Fig.6. The effect of a γ-aminobutyric acid subunit A (GABA-A) receptor antagonist bicuculline (1 mg/kg i.p), on apelin-induced (2 µg/2 µl i.c.v) consolidation of passive avoidance learning. . Data are expressed as means ± S.E.M. Number in bars are the numbers of animals used.

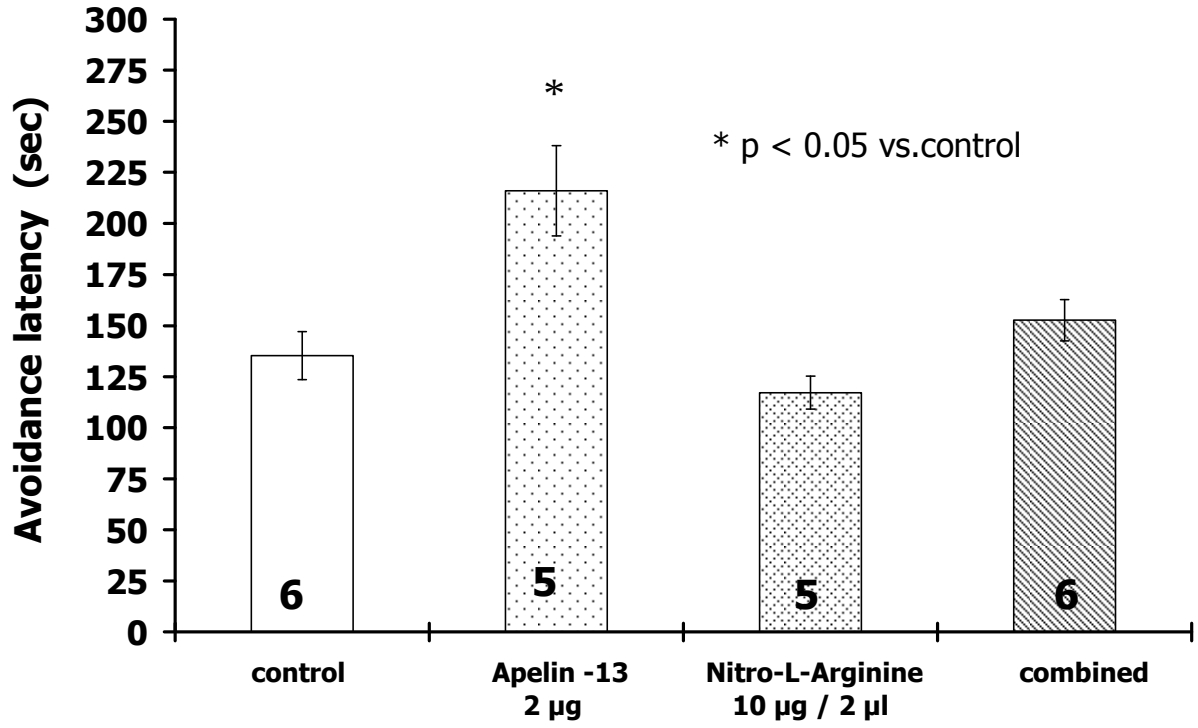

Fig.7.

Fig.7. The effect of a nitric oxide (NO) synthase inhibitor, nitro-L-arginine (10 µg/ 2µl) on apelin-induced(2 µg/2 µl i.c.v) consolidation of passive avoidance learning. Data are expressed as means ± S.E.M. Number in bars are the numbers of animals used.