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Dopamine D4 receptor antagonist L745,870 abolishes cognitive effects of intracerebroventricular angiotensin IV and des-Phe^6-Ang IV in rats

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Abstract

In this study effect of L745,870, a selective D_4 dopamine (DA) receptor blocker, on the pro-cognitive action of intracerebroventricularly (icv) injected angiotensin IV (Ang IV) and des-Phe^6-Ang IV was examined. Male Wistar rats weighing 180–200 g were used. Both peptides given at the dose of 1 nmol facilitated recall of a passive avoidance (PA) behaviour, improved object recognition (OR) memory, decreased number of errors, increased number of sequential correct entries and shortened time-to-goal in an eight-arm radial maze (RM). In the auxiliary tests performed to control for the participation of unspecific motor (open field, OF) and emotional ('plus' maze, PM) effects of our treatment in the results of memory tests they had either no (OF) or negligible (PM) effects. Intraperitoneal pretreatment of the animals with 1 mg/kg of L745,870 abolished effects of both peptides on PA and OR and slightly diminished those observed in the eight-arm RM.

1. Introduction

Although pro-cognitive effects of the octapeptide angiotensin IV (Val-Tyr-Ile-His-Pro-Phe-OH, Ang IV) have been known for 20 years (Braszko et al., 1988) they are still far from being understood. Obviously, whatever the mechanisms of these effects they must be integrated with a complex central nervous system (CNS) information processing involving several other neuroactive systems including cholinergic, glutamatergic, dopaminergic and some peptidergic for example opioid, neurohypophyseal, and other (for review see Decker and McGaugh, 1991).

In the abovementioned early study (Braszko et al., 1988) we found that Ang IV along with facilitating learning of active and recall of passive avoidances, potently enhanced motor stereotypy, a well known dopamine (DA) receptor mediated behaviour (Randrup and Munkvad, 1974). Because DA involvement in the Ang IV cognitive enhancement appeared likely we chose to systematically search for it by addressing the question how inhibition of the particular DA receptor subpopulations D_{1-3} (Braszko, 2004, 2006; Braszko et al., 2008) with the highly selective blockers influences pro-cognitive activity of Ang IV. To facilitate comparisons we have used a battery of roughly the same behavioural tests: recall of a passive avoidance (PA)

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behaviour (Ader et al., 1972), learning of conditioned avoidance responses (CARs) (Braszko et al., 1987), object recognition (OR) test (Ennaceur and Meliani, 1992) and eight-arm radial maze (RM) for the cognitive testing.

Since motor and emotional effects of the peptides as well as those of the receptor blockers may considerably bias results of the cognitive tests we also ran auxiliary experiments searching for these unspecific effects in respectively, open field (OF) (Braszko et al., 1987) and elevated 'plus' maze (PM) (Pellow et al., 1985).

Because of the considerable angiotensin AT_1 receptor activity of Ang IV (Le et al., 2002) we also examined parallel groups of rats injected with des-Phe^6-Ang IV having similar to Ang IV AT_4 receptor affinity (Sardinia et al., 1993) and behavioural activity (Braszko et al., 1991) but devoid of cardiovascular and other AT_1 effects.

Our previous studies demonstrated that D_1 and D_2 but not D_3 dopamine receptor blockade abolishes or considerably attenuates pro-cognitive effects of both peptides (Braszko, 2004, 2006; Braszko et al., 2008).

In the present study it is shown that the compound L-745,870, a potent and highly selective dopamine D_4 receptor inhibitor (Patel et al., 1997) makes Ang IV and des-Phe^6-Ang IV unable to improve some but not all aspects of information processing in rats.

2. Experimental procedures

2.1. Animals

All experiments were conducted on male Wistar rats (160–180 g) housed five to a cage in a temperature controlled room (22°C) on a 12 h:12 h light/dark cycle beginning at 07:00 h. They had free access to standard laboratory food and tap water. All animals were handled daily for 3 min each until the day of experiment. The experiments were conducted between 09:00 and 14:00 h. A 30 min adaptation period in the experimental room preceded all tests. All experiments were approved by the Local Ethics Commission for Animal Experimentation.

2.2. Surgery

Under the ether anaesthesia a round piece of skin, about 7 mm in diameter, was cut out of the top of head and underlying bone cleaned of soft tissue. A burr hole, 0.5 mm in diameter was drilled in the skull 2.5 mm laterally and 1 mm caudally from the point of intersection of bregma and the superior sagittal suture on the right side of the head. The operation took about 2 min and, after 72 h recovery, the wound was completely dry and the animal behaved normally. Intracerebroventricular (icv) injections were made freehand into the right cerebral ventricle with a 10 μl Hamilton syringe, using a KF 730 needle cut 4.5 mm from its base. In the procedure the tip of the needle was lowered about 0.5 mm below the ceiling of the lateral cerebral ventricle without touching its bottom. The whole intervention was relatively non-traumatic as the animal, gently fixed by the bare hand of the experimenter, was usually quiet and no vocalization occurred. The injection volume was always 2 μl administered slowly (3 s). After the experiments all rats were sacrificed and the sites of injection were verified microscopically after brain sectioning.

2.3. Passive avoidance

Passive avoidance (PA) behaviour was studied in a one-trial learning, step-through situation, which utilizes the natural preference of rats for a dark environment (Ader et al., 1972). The experiment was conducted exactly as described previously (Braszko et al., 2006).

2.4. Object recognition

Object recognition (OR) was tested in a plastic box 62 cm long, 38 cm wide and 20 cm high covered with a wire mesh lid. The objects to be discriminated were made of glass or porcelain and existed in duplicate. They appeared to have no natural significance for the rats and they had never been associated with reinforcement. Their weight was such that they could not be displaced by the rats. The procedure was similar to that described previously (Ennaceur and Meliani, 1992) and may be summarized as follows. All rats were submitted to two habituation sessions, with a 1-h interval, whereby they were allowed 3 min exploration of the apparatus. Twenty-four hours later testing began. The experimental session consisted of two trials, each lasting for 3 min. In the first trial (T1), rats were exposed to two identical objects A. In the second trial (T2), performed 60 min later, rats were exposed to two objects, one of which was duplicate of familiar object A (A′) in order to avoid olfactory traits, and a new object B. From rat to rat, the role (familiar or new object) as well as the relative position of the two objects were counterbalanced and randomly permuted during trial T2. These precautions were taken in order to reduce object and place preference effects. The basic measure was the time spent by the rat in exploring objects during trials T1 and T2. Exploration of an object was defined as touching it with the nose. Turning around or sitting on the object was not considered exploratory behaviour. From this measure, the following variables were defined: *A*, the time spent in exploring, objects *A* in T1; *A′* and *B*, the times spent in exploring respectively, the duplicate of familiar, and the new object in T2. Object recognition was measured by the variable $B-A'$, and total exploration in T2 by $B+A'$. Moreover, as $B-A'$ may be biased by the differences in the overall levels of exploration, the variable $B-A' / B+A'$ was also computed.

2.5. Eight-arm radial maze

The apparatus was an elevated eight-arm radial maze which was made of 0.5 cm gray wooden planks. Each arm (68×10 cm) extended from an octagonally shaped center hub (30 cm in diameter). A small cup (4.5 in diameter) was placed at the end of each arm. The maze was elevated 80 cm above the floor and placed in a sound attenuated room with a masking noise of 70 dbs above the human thresholds (Ennaceur and Meliani, 1992). The rats were submitted to three habituation sessions (one per day). They were put on the central platform and allowed to explore the maze for 10 min. At the end of the habituation stage, they were deprived of water for 48 h before the beginning of the training phase. Rats were given 12 sessions (one per day). The training sessions lasted until the rats made eight choices or until 15 min elapsed. The cups at the end of each arm contained 0.3 ml of water. The experimenter recorded the order of the eight choices made by each rat. One hour after the completion of the session, rats were given water for 10–15 min. Three following measures of rat's behaviour were considered: (i) number of errors: an error was an entrance in a previously visited arm, (ii) number of correct (made in sequence) choices before the first error in each session and (iii) time used up for visiting all eight arms.

2.6. Open field

Locomotor exploratory activity was measured in an open field which was a square 100 cm×100 cm white floor divided by eight lines into 25 equal squares and surrounded by a 47 cm high wall (Braszko et al., 1987).

2.7. Elevated 'plus' maze

Anxiety was evaluated in an elevated 'plus' maze (constructed of grey coloured wooden planks) consisting of two open arms, 50 cm