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INFLUENCE OF INTRACEREBROVENTRICULAR INJECTED 6-OHDA ON CARDIOVASCULAR EFFECTS OF ACETYLCHOLINE, PILOCARPINE AND NICOTINE

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Summary. *The influence of catecholamine synthesis inhibitor, 6-OHDA on cardiovascular effects of intracerebroventricularly (i.c.v.) injected acetylcholine, pilocarpine and nicotine was investigated in anaesthetized cats. Acetylcholine, pilocarpine and nicotine administered i.c.v. caused a dose-dependent decrease of blood pressure. In animals previously treated with inhibitor synthesis of catecholamine, 6-OHDA, a statistically significant fall or abolition of hypotensive effects acetylcholine, pilocarpine and nicotine were obtained. Based on these results the authors suggest that the intact central catecholaminergic neuron is needed for nicotinic and muscarinic receptors in the central regulation of arterial blood pressure.*

Key words: *Hypotensive activity, acetylcholine, pilocarpine, nicotine, 6-hydroxidopamine*

Introduction

By injecting the intracerebroventricular (i.c.v.) muscarinic and nicotinic agonists, various cardiovascular effects can be recorded (1-3). For instance, acetylcholine administered intracerebroventricularly in anaesthetized and non-anaesthetized dogs produces a transient increase of blood pressure (4), whereas in rats increases the blood pressure (5, 6). Moreover, in anaesthetized cats acetylcholine microinjected in different brain structures causes pressor or depressor effects (7, 8). In addition it has been demonstrated that nicotine injected i.c.v. in anaesthetized cats induced a fall of arterial blood pressure (9). On the other hand, the reports on the effects of pilocarpine on the central regulation of arterial blood pressure are still scarce.

It was shown that cardiovascular effects obtained by stimulation of muscarinic and nicotinic receptors were missing in those animals which were treated with reserpine (10, 11). In addition it is well known that reserpine depletes the stores of catecholamines,

5-hydroxytriptamine and acethylcholine. Because of that, in the present experiments the predominantly inhibitor of catecholamine synthesis 6-OHDA was used.

The aim of this study was to establish if the cardiovascular effects caused by central stimulation of muscarinic and nicotinic receptors were accomplished with intact cateholaminergic neuron participation. In the present experiments the predominantly inhibitor of catecholamine synthesis 6-OHDA was used. Therefore the role of muscarinic and nicotinic receptors in the central catecholaminergic neuron in the central regulation of blood pressure was investigated by injecting acethylcholine, pilocarpine and nicotine into the cerebral ventricles in cats treated with 6-OHDA similarly administered.

Materials and methods

Subjects

Male and female cats, weighting between 2 and 3.5 kg were used in this study. The cats were housed individually in stainless steel cages (80 cm × 60 cm × 60 cm) under standard laboratory conditions. All experimental procedures with animals were in compliance with The European Council Directive of November 245, 1986 (86/609/EC)

Surgical procedures

Each animal was anaesthetized using pentobarbital sodium (40 mg kg^{-1} i.p.). Following aseptic precaution, a hole was drilled 7-8 mm from the stereotaxic zero line and 4-5 mm from the midline. A Collison cannula was then screwed into the calvarium, so that the tip of the cannula rested in the left lateral ventricle (for details see: Veljković et al. 1989). The lower end of the shaft of the cannula was made of polyethylene tubing with a side opening 1 mm from its closed tip and positioned with the lumen facing the foramen of Monro. Postoperatively, penicillin was administered intramuscularly. An interval of five days elapsed after surgery before an experiment was started. Post-mortem studies indicated that the injected material passed from the lateral ventricle into the third and fourth ventricle.

6-hydroxydopamin 1 mg of dose was injected on the 13th, 12th and 11th day before the experiments. The solution of drugs was injected by hand in a volume 0.1 ml over a period of 15-20 seconds and washed in with 0.1 ml of 154 mM solution of NaCl.

Testing procedures

The arterial blood pressure was recorded on a kymograph from the cannulated left carotid artery, connected with a mercury manometer. The mean blood pressure was estimated as (systolic + 2 diastolic blood pressure) / 3. The heart rate was recorded electrocardiographically.

Drugs

The compounds used in these experiments were: 6-hydroxidopamine bromide, acetylcholin iodide, pilocarpine and nicotine bitartarate. The drugs were dissolved in 154 mM solution of NaCl. The doses of drugs refer to the salts.

Statistics

All data are presented as means of 7-8 experiments ± s.e.m. Calculations of the mean effective doses ID50 and their 95% confidence limits were calculated using linear regression according to the methods of least squares. ID50 is the dose required to produce 50% of the maximal depressor response to i.c.v. acetylcholine, pilocarpine and nicotine in control experiments and in the presence of 6-OHDA. Changes of registered parameters were estimated by 1-way analysis of variance.

Results

Intracerebroventricular administration of acetyilcholine, pilocarpine and nicotine

In anaesthetized cats value of basal mean arterial blood pressure was 132+8.8 mmHg, and heart rate was 177 +10.8/min

Acetyilcholine (0.01-0.3mg; r=0.98, P<0.05), pilocarpine (0.3-1 mg; r = 0.99, P<0.01) and nicotine (0.01-0.2 mg; r = 0.994, P<0.01) administered intracerebroventricularly induced a dose-dependent hypotension. Used drugs did not change heart rate (not shown).

Effect of 6-OHDA of arterial blood pressure response on acetyilcholine, pilocarpine and nicotine

After injecting 6-OHDA, the arterial blood pressure and heart rate values were 131.6 ± 10.1 mmHg 157.1 ± 11.9 /min. Intracerebroventricularly injected 6-OHDA completely diminished hypotensive effects of acetylcholine (Fig. 1), pilocarpine (Fig. 2) and significantly decreased hypotensive effect of nicotine (Fig. 3).

Discussion

Our present experiments show that acetylcholine, pilocarpine and nicotine injected into the cerebral ventricles of anaesthetized cats significantly reduced the arterial blood pressure. This is in agreement with the findings of other authors (1, 9, 12). Moreover, it is interesting to note that DMPP, agonist of nicotinic receptors, injected i.c.v., does not change the arterial blood pressure to a great degree (13). Because acetylcholine and pilocarpine are muscarinic agonists, and nicotine and DMPP nicotinic agonists, it could be rightfully affirmed that they are acting predominantly via central muscarinic and nicotinic receptors. However, since the differences in effects of DMPP and nicotine do exist, it could be presumed that nicotine and DMPP don't act by the same nicotinic receptors. This presumption is maintained by the findings which show that the nicotine, not DMPP, provokes the relaxation of the circular strips from cat's stomach (14).

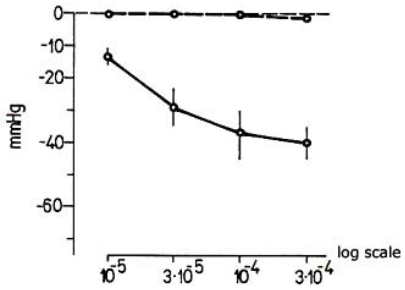

Fig. 1. The effect of 6-OHDA on hypotensive responses to acetylcholine injected into cerebral ventricles (- - - -) of anaesthetized cats. The full line is control (——). Ordinate: fall of blood pressure. Abscissa: doses of acetylcholine in mg.

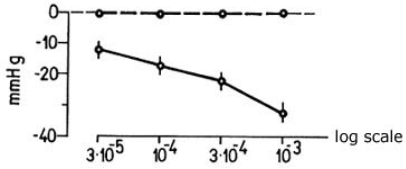

Fig. 2. The effect of 6-OHDA on hypotensive responses to pilocarpine injected into cerebral ventricles (- - - -) of anaesthetized cats. The full line is control (——). Ordinate: fall of blood pressure. Abscissa: doses of pilocarpine in mg.

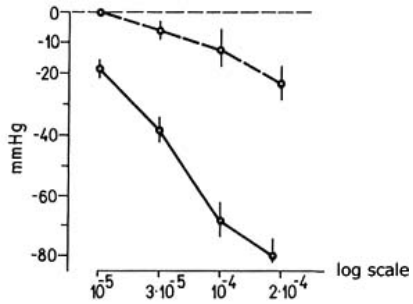

Fig. 3. The effect of 6-OHDA on hypotensive responses to nicotine injected into cerebral ventricles (- - - -) of anaesthetized cats. The full line is control (——). Ordinate: fall of blood pressure. Abscissa: doses of nicotine in mg.

In cats treated with reserpine i.c.v, the cardiovascular effects provoked by muscarinic and nicotinic receptors central stimulation were absent. Since, the i.c.v. application of reserpine empties catecholaminergic and cholinergic storages, it is not possible to establish whether the present effects in reserpined animals occurred as a consequence of cholinergic or catechol-

aminergic neuron damage.

In our experiments, the pretreatment with 6-OHDA abolished or significantly reduced cardiovascular effects provoked by injected acetylcholine, pilocarpine and nicotine. Biogenic amines containing nerve cells and terminals have been identified in the brainstem (15). These results show that for the expression of the mentioned muscarinic and nicotinic agonists effects the intact catecholaminergic neuron is necessary.

It is well known that the applied agonists express their cardiovascular effects by central muscarinic and nicotinic receptors stimulation (1, 2). However, our experiments can not conclude in which places of catecholaminergic neuron this receptors exist.

The intact catecholaminergic neuron is needed not only for blood pressure regulation (1, 13), but for behavior and body temperature regulation (16, 17).

Based on the above results, it can be concluded that the intact catecholaminergic neuron is necessary for the expression of cardiovascular effects caused by central stimulation of muscarinic and nicotinic receptors.

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UTICAJ INTRACEREBROVENTRIKULARNO UBRIZGANOG 6-OHDA NA KARDIOVASKULARNE EFEKTE ACETIL HOLINA, PILOKARPINA I NIKOTINA

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Kratak sadržaj: *Praćen je uticaj inhibitora sinteze kateholamina, 6-OH DA na kardiovaskularne efekte intracerebroventrikularno ubrizganih acetilholina, pilokarpina i nikotina. Intracerebroventrikularno ubrizgani acetilholin, pilokarpin i nikotin, izazvali su dozno zavisno sniženje krvnog pritiska. U životinja prethodno tretiranih inhibitorom sinteze kateholamina, 6-OHDA registrovan je statistički značajno manji (do odsutni) hipotenzivni efekat acetilholina, pilokarpina i nikotina. Na osnovu dobijenih rezultata autori zaključuju da se kardiovaskularni efekti izazvani centralnom stimulacijom nikotinskih i muskarinskih receptora ostvaruju uz učešće intaktnog kateholaminergičkog neurona.*

Ključne reči: *Hipotenzivno dejstvo, acetilholin, pilokarpin, nikotin, 6-hidroksidopamin*