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Central Cholinergic Mechanisms in L-DOPA Induced Hyperactive Urinary Bladder of the Rat

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Summary. The involvement of central and peripheral cholinergic structures in the mediation of a centrally induced hyperactive urinary bladder response to L-2,3-dihydroxyphenylalanine (L-DOPA), after peripheral decarboxylase inhibition, registered by a cystometric procedure, has been analysed pharmacologically in anaesthetised rats. The urinary bladder response to L-DOPA was unchanged after blockade of cholinergic receptors with methylscopolamine, diminished after atropine and totally inhibited after hexamethonium. In addition, activation of muscarinic receptors in the pontine-mesencephalic brain region with oxotremorine after methylscopolamine pretreatment generates a hyperactive urinary bladder response, mediation of which seems to be independent of endogenous catecholamine stores. It is suggested that cholinergic receptors in the pontine-mesencephalic brain region are of importance for regulation of urinary bladder function in the rat. Furthermore, the bladder hyperactivity induced by L-DOPA might be propagated via muscarinic receptors in this brain area, and mediated peripherally via cholinergic receptors in the autonomic ganglia, but in the bladder detrusor via non-cholinergic receptors.

Key words: Central muscarinic receptors, L-DOPA, Cholinergic mediation, Urinary bladder.

Introduction

Central nervous transmitter control mechanisms for urinary bladder function are complicated and little understood. The importance of central monoaminergic control mechanisms has been discussed [1, 2] but no experimental data are available. Recently, however, we have shown that activation of central monoamine neurones with L-DOPA can influence urinary bladder function in the rat [3]. The hyperactive urinary bladder reaction thus evoked appears to be elicited via dopaminergic receptors [4] in the pontine-mesencephalic region in the brain [5], and seems to be mediated to the bladder via the pelvic nerves [5]. An interaction between central adrenergic and cholinergic mechanisms is indicated by the fact that the action on the bladder is generated in central catecholamine structures and mediated via peripheral cholinergic pathways.

In the present study the above-proposed interaction is further analysed. The urinary bladder response is studied after activation of central cholinergic structures with an without depletion of central CA stores, and after blockade of central and/or peripheral cholinergic mechanisms during L-DOPA stimulation.

Methods

Male Sprague-Dawley rats weighing 180–250 g were used in all experiments. The rats were anaesthetised with pentobarbitone sodium (Nembutal) 60 mg/kg. The proximal urethra was exposed and incised via a suprapubic incision and a catheter was inserted into the bladder. The catheter was secured with a ligature around the proximal urethra. In all experiments, the pressure response of the bladder to a continuous volume increment was studied, i.e. a cystometric examination was performed. The bladder was filled with saline at room temperature at a rate of 2.0–3.3 ml/h from a constant volume infusion pump. The intravesical pressure was simultaneously recorded with a Statham pressure transducer (*P* 23 Dc) on a Grass-Polygraph. The infusion was continued until an intravesical pressure of 25 mmHg was attained. Cystometry was performed before and after injection of the drugs used in the experiments in all animals. No detrusor contractions were observed during control cystometric recording in anaesthetised rats, i.e. before injection of drugs.

In a few rats brain transection was performed at the level just below the inferior colliculi, i.e. infracollicular decerebration. In another series of experiments decerebration was performed at the level of the superior colliculi (for further details, see Sillén et al. [5]). The lesions were inflicted under diethylether anaesthesia 1 h before cystometric recordings.

Drugs

The following drugs were used: pentobarbitone (sodium form, Mebumal Vet., ACO, Solna, Sweden), α-hydrazinomethyl-β-(3,4-

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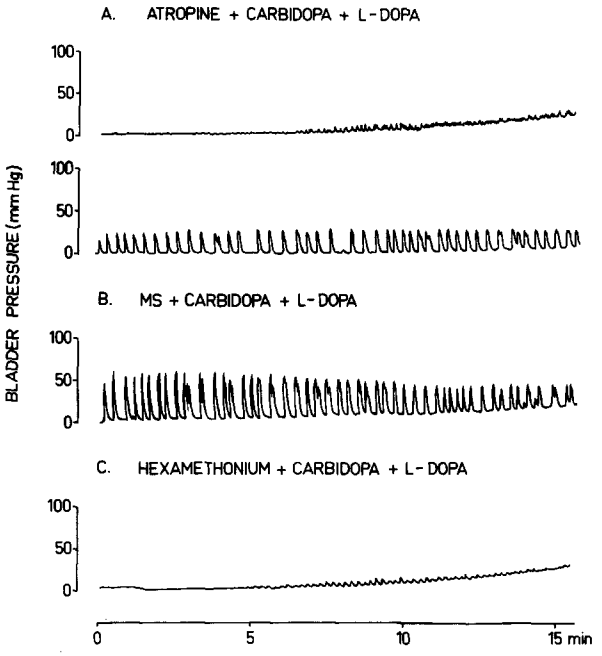

Fig. 1. Effects on the rat urinary bladder of L-DOPA, after peripheral decarboxylase inhibition, and blockade of cholinergic receptors with atropine, methylscopolamine (MS) and hexamethonium. The curves are cystometric recordings (mmHg) during constant infusion of saline (3.3 ml/h) in anaesthetised rats, after i.p. injections of *A*. atropine (5 mg/kg, 40 min), carbidopa (100 mg/kg, 30 min) and L-DOPA (100 mg/kg, 15 min), *B*. MS (5 mg/kg, 40 min), carbidopa (100 mg/kg, 30 min) and L-DOPA (100 mg/kg, 15 min), and *C*. hexamethonium (10 mg/kg, 40 min), carbidopa (100 mg/kg, 30 min) and L-DOPA (100 mg/kg, 15 min). Curves *B* and *C* are typical examples of events that occurred regularly in all rats. The curves in *A* represent two different bladder reactions to L-DOPA after atropine pretreatment. The number of experiments is given in the text

dihydroxyphenyl) propionic acid (MK-486, carbidopa, Merck, Sharp & Dohme, Rahway, U.S.A.), L-3,4-dihydroxyphenylalanine (L-DOPA, Astra, Södertälje, Sweden), reserpine (CIBA, Mölndal, Sweden), DL-α-methyltyrosine methylester HCL (α-MT, H 44/68, Hässle, Mölndal), guanethidine sulph. (Ismelin®, CIBA), Sotalol (Mead Johnson, Evansville, Indiana U.S.A.), propranolol (Inderal®, ICI, Göteborg, Sweden), phentolamine (Regitin®, CIBA), N-methylscopolamine nitrate (Pharmacia, Uppsala, Sweden), atropine sulphate (Sigma, St. Louis, U.S.A.), hexamethonium chloride (Fluka, Buchs, Switzerland) and oxotremorine (Aldrich, Beerse, Belgium).

Results

Effects of Blockade of Cholinergic Receptors with Atropine Methylscopolamine and Hexamethonium on the Bladder Hyperactivity Induced by L-DOPA

Central adrenergic stimulation with L-DOPA (100 mg/kg i.p.), after peripheral decarboxylase inhibition with carbidopa (100 mg/kg i.p., 15 min before L-DOPA), resulted within 15 min in prominent detrusor contractions (for further details, see Sillén et al. [3]). The possibility that cholinergic neurones are involved in the mediation of the bladder response to central adrenergic stimulation (cf. Introduction) was studied by blockade of cholinergic receptors during L-DOPA stimulation. Experiments were performed to test whether the bladder hyperactivity to L-DOPA could be inhibited by blockade of A) peripheral muscarinic rceptors in the bladder detrusor with methylscopolamine, B) peripheral nicotinic receptors in the vesical ganglia with hexamethonium, and C) both peripheral and central muscarinic receptors with atropine.

Methylscopolamine pretreatment (1 mg/kg (n = 6), 5 mg/kg (n = 6), 10 mg/kg (n = 6) and 20 mg/kg (n = 6)), administered i.p. 10 min before carbidopa (Fig. 1), did not prevent the hyperactive urinary bladder response to L-DOPA in any case. The amplitude of the detrusor contractions, however, seemed to be somewhat reduced compared with those seen after L-DOPA alone.

Hexamethonium (10 mg/kg i.p., (n = 6), 10 min before carbidopa, (Fig. 1) completely prevented the detrusor contractions in response to L-DOPA in all animals.

Atropine pretreatment had an inconsistent inhibitory action on the hyperactive urinary bladder response to L-DOPA. Administration of atropine, 1 mg/kg (n = 10), 5 mg/kg (n = 14), 10 mg/kg (n = 10) and 20 mg/kg (n = 10), i.p., 10 min before carbidopa, Fig. 1, totally inhibited the hyperactive bladder in response to L-DOPA in none (1 mg/kg), six (5 mg/kg), four (10 mg/kg) and four (20 mg/kg) animals, while in the remaining animals detrusor contractions were seen, but with lower amplitude than those seen after L-DOPA alone.

Effects of Central Cholinergic Stimulation with Oxotremorine on Animals with Intact and Depleted CA Stores

To investigate further the possible involvement of central cholinergic mechanisms in the generation and mediation of a hyperactive urinary bladder response, central cholinergic activation was performed in animals with intact CA stores, and in animals in which the CA stores had been depleted with reserpine and α-methyltyrosine (α-MT). The central cholinergic activation was achieved by injecting oxotremorine, a muscarinic agonist known to penetrate the blood-brain barrier [6], after blockade of peripheral muscarinic receptors with methylscopolamine (MS).

Oxotremorine and MS. Central cholinergic activation with oxotremorine (0.5 mg/kg i.p., n = 10) after pretreatment with MS (5 mg/kg i.p., 15 min before oxotremorine), resulted within 20–30 min in a hyperactive bladder response in all animals (Fig. 2). The amplitude and frequency of the detrusor contractions, however, seemed to be somewhat reduced compared with those seen after L-DOPA. With a lower dose of oxotremorine (0.1 mg/kg i.p., n = 6) the response was inconsistent (3 out of 6).

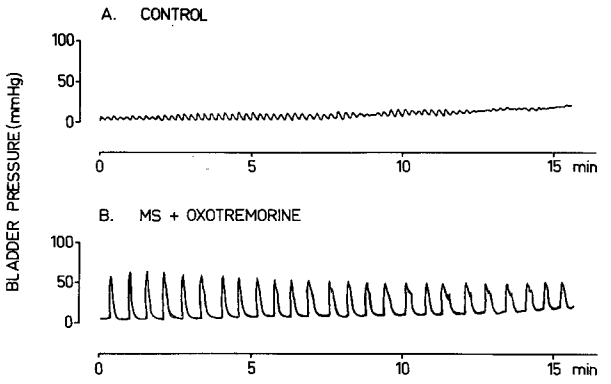

Fig. 2. Effects on the rat urinary bladder of central cholinergic stimulation with oxotremorine, after pretreatment with methyl-scopolamine (MS). The curves are cystometric recordings (mmHg) during constant infusion of saline (3.3 ml/h) in anesthetised rats. *A*. Control, i.e. without any pretreatment, and *B*, after i.p. injections of MS (5 mg/kg, 45 min) and oxotremorine (0.5 mg/kg, 30 min). The curves are typical examples of events that occurred regularly in all rats

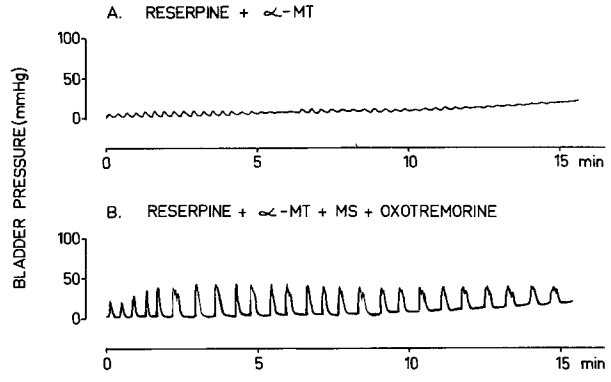

Fig. 3. Effects on the rat urinary bladder of central cholinergic stimulation with oxotremorine after depletion of endogenous catecholamine stores with reserpine and α-methyltyrosine (α-MT). The curves are cystometric recordings (mmHg) during constant infusion of saline (3.3 ml/h) in anaesthetized rats, after i.p. injections of reserpine (10 mg/kg, 12 h) and α-MT (250 mg/kg, 4 h). *A*. without additional pretreatment, and *B*. with additional i.p. injections of methylscopolamine (MS, 5 mg/kg, 45 min) and oxotremorine (0.5 mg/kg, 30 min). The curves are typical examples of events that occurred regularly in all rats

The central origin of the bladder response to oxotremorine was established by subjecting six animals to an infracollicular transection of the brain before injecting the drug. In all these animals no detrusor contractions occurred after MS and oxotremorine. When six animals were subjected to transection at a higher level of the brain, i.e. at the superior colliculi, however, detrusor contractions occurred in all animals after MS and oxotremorine.

In order to show that the bladder reaction to oxotremorine really was due to activation of central muscarinic receptors, stimulation experiments were performed under

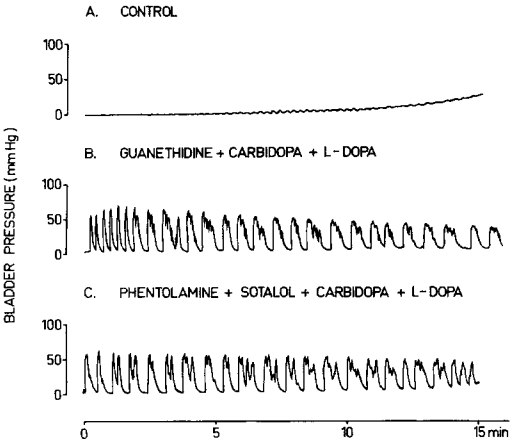

Fig. 4. Effects on the rat urinary bladder of L-DOPA, after peripheral decarboxylase inhibition, and blockade of peripheral adrenergic mechanisms with guanethidine, phentolamine and sotalol. The curves are cystometric recordings (mmHg) during constant infusion of saline (3.3 ml/h) in anaesthetised rats. *A*. Control, i.e. without any pretreatment. *B* and *C* after i.p. injections of: *B*. guanethidine (15 mg/kg, 4 h), carbidopa (100 mg/kg, 30 min) and L-DOPA (100 mg/kg, 15 min) and *C*. phentolamine (5 mg/kg, 75 min), sotalol (2 mg/kg, 75 min), carbidopa (100 mg/kg, 30 min) and L-DOPA (100 mg/kg, 15 min). The curves are typical examples of events that occurred regularly in all rats

blockade of both peripheral and central muscarinic receptors by pretreatment with atropine. Atropine 20 mg/kg ($n = 6$), administered i.p. 5 min before oxotremorine, inhibited the hyperactive bladder response to oxotremorine in all animals tested, whereas lower doses of atropine (5–10 mg/kg, $n = 6$) gave an inconsistent bladder response.

Reserpine, α-MT, Oxotremorine and MS. To test whether the effect on the bladder of central cholinergic activation with oxotremorine after MS pretreatment was dependent on intact CA neurons or not, stimulation experiments were performed in animals in which endogenous CA stores had been depleted. Depletion of endogenous CA stores was achieved by inhibition of the CA uptake in the nerve terminal storage granules with reserpine [7], combined with inhibition of the CA synthesis with α-MT [8]. Pretreatment with reserpine (10 mg/kg i.p., 12 h before MS) and α-MT (250 mg/kg i.p., 4 h before MS) did not alter the bladder response to oxotremorine (0.5 mg/kg i.p., $n = 8$, Fig. 3) in comparison with the effects observed after the drug in animals with intact CA stores.

Effects of Blockade of Peripheral Adrenergic Mechanisms with Sotalol, Phentolamine and Guanethidine on the Bladder Hyperactivity Induced by L-DOPA

The importance of peripheral adrenergic receptors and pathways in the mediation of the hyperactive bladder response

to central adrenergic activation with L-DOPA was investigated by blockade of the α- and β-receptors in the urinary bladder with phentolamine and sotalol, respectively. In some experiments propranolol was used instead of sotalol. In another series of experiments peripheral adrenergic neurones were blocked by administration of guanethidine [7].

Phentolamine and Sotalol/propranolol. Pretreatment with phentolamine (5 mg/kg i.p., 30 min before carbidopa) and sotalol or propranolol (2 mg/kg i.p., 30 min before carbidopa) did not influence the hyperactive urinary bladder response seen after central adrenergic stimulation with L-DOPA (n = 8, Fig. 4).

Guanethidine pretreatment (15 mg/kg i.p., n = 8,5 h before carbidopa, Fig. 4) did not prevent the detrusor contractions seen after stimulation with L-DOPA.

Discussion

Activation of central cholinergic receptors with the muscarinic agonist oxotremorine, after blockade of peripheral muscarinic receptors with methylscopolamine, results in a hyperactive urinary bladder response in the rat. Transection experiments indicate that this bladder response originates in the pontine-mesencephalic brain region, an area known to be fairly rich in cholinergic structures [9].

When central adrenergic mechanisms were activated with the CA precursor L-DOPA, after peripheral dopa-carboxylase inhibition, a similar bladder response was obtained [3]. This action has also been localised to pontine-mesencephalic structures [5]. Thus, it appears possible to induce a hyperactive bladder response after either central adrenergic or central cholinergic activation in this region. These findings suggest that the two transmitter systems activate similar motor phatways to the bladder, possibly indicating an interaction between adrenergic and cholinergic mechanisms supraspinally.

The close relationship between adrenergic and cholinergic substances in the brain with respect to metabolism and behavioural functions is well documented [10]. A central interaction between adrenergic and cholinergic mechanisms concerning regulation of autonomic functions, as proposed in this paper, has also been discussed but few experimental data are available. Some support for this hypothesis is provided by the finding that central dopaminergic mechanisms influence vagal activity in the ventricle, as shown by Uvnäs et al. [11].

Some of the results in this study might indicate a central interaction with cholinergic receptors in the mediation of the bladder response to central adrenergic stimulation. Such results are the findings that the bladder hyperactivity induced by L-DOPA could be inhibited in some experiments by blockade of central muscarinic receptors with atropine, and that the bladder response to L-DOPA could be mimicked by stimulation of central muscarinic receptors with oxotremorine. A central action of atropine in the above-described experiments is indicated by the finding that the atropine analogue methylscopolamine which does not cross the blood-brain barrier, had no inhibitory effect on bladder hyperactivity induced by L-DOPA. The above-suggested interaction ought to take place in the pontine-mesencephalic brain region, since there seem to be no cholinergic receptors in the central part of the motor pathways to the bladder below this area. This hypothesis is based on the fact that stimulation of central muscarinic receptors with oxotremorine had no effect on the bladder in animals transected below this region, but a response was seen after transection above this area.

On the other hand, there seems not to be an interaction with central adrenergic neurones in the mediation of the bladder response to central cholinergic stimulation with oxotremorine, since the effect was unchanged after depletion of endogenous transmitter stores in the catecholamine neurons with reserpine and α-MT.

As mentioned in the introduction, the peripheral mediation of the bladder response to central adrenergic stimulation is propagated via the pelvic nerves, and thus thought to be cholinergic in nature. This seems to be true for the mediation via the preganglionic fibres in the pelvic nerve, since the transmission of the bladder hyperactivity in the pelvic autonomic ganglia could be inhibited by hexamethonium, an agent known to block cholinergic receptors in peripheral ganglia. However, contrary to expectations, the transmitter in the urinary bladder responding to stimulation of these excitatory motor pathways appears to be mainly noncholinergic, since the bladder response was unchanged after blockade of peripheral muscarinic receptors. Furthermore, it appears to be non-adrenergic as blockade of peripheral adrenergic receptors had no effect.

Although the motor response in the bladder, mediated via the pelvic nerves, is thought to be cholinergic in nature, muscarinic receptor blocking agents, such as MS and atropine, are known to inhibit nerve-mediated detrusor contractions incompletely. Many theories have been put forward to expain this atropine resistence of the bladder (for a review, see Elmér [12]). The most plausible explanation seems to be the presence of another noncholinergic excitatory transmitter system in the urinary bladder. Several possible transmitter substances have been proposed, including peptides [13–16], adenosinetriphosphate (ATP, i.e. purinergic nerves [17, 18], prostaglandins [19] and histamine [20, 21].

In conclusion, the findings in this study show that the urinary bladder motor pathways, originating in the pontine-mesencephalic region, can be excited by central adrenergic as well as cholinergic receptor stimulation. Thus, both these transmitter systems seem to be of importance for the central regulation of bladder function. Furthermore, the peripheral mediation of the centrally induced hyperactive bladder response to L-DOPA seems to be mediated via the pelvic nerves; in the vesical ganglia via cholinergic mechanisms but postganglionically, i.e. in the bladder detrusor, mainly via non-cholinergic receptors.

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