Modulation by the muscarinic agonist McN-A-343 of release of noradrenaline from sympathetic neurones in the rabbit pulmonary artery

O.A. NEDERGAARD

Department of Pharmacology, Odense University, J.B. Winslows Vej 19, DK-5000 Odense C, Denmark

McN-A-343 (4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethyl-ammonium chloride) is a powerful muscarinic agonist on sympathetic ganglion cells (Roszkowski, 1961). In this study its effect on the overflow of tritium released by electrical-field stimulation (225 mA; 300 and 1000 pulses; 0.5 msec; 3 Hz) was studied on the rabbit isolated pulmonary artery preloaded with (−)-[^{3}H]-noradrenaline ([^{3}H]-NA).

The time course of the effect of McN-A-343 and cocaine on overflow of tritium evoked by stimulation was examined. At 10^{-6}–3×10^{-5} M, McN-A-343 continuously enhanced the [^{3}H]-overflow up to 192% of control. At higher concentrations (10^{-4} and 3×10^{-4} M), McN-A-343 initially potentiated the overflow and thereafter inhibited it. Cocaine in the lower concentrations (10^{-6}–3×10^{-5} M) also enhanced the [^{3}H]-overflow evoked by stimulation, but to a lesser degree (maximally 126% of control) than that seen with McN-A-343. At higher concentrations (10^{-4} and 3×10^{-4} M), cocaine solely reduced the [^{3}H]-overflow.

The spontaneous outflow of tritium from pulmonary artery preloaded with [^{3}H]-noradrenaline ([^{3}H]-NA) consisted of [^{3}H]-NA (13%), [^{3}H]-dihydroxyphenyl ethyl glycol ([^{3}H]-DOPEG, 17%), [^{3}H]-dihydroxy mandelic acid ([^{3}H]-DOMA, 8%), [^{3}H]-O-methylated and deaminated metabolites ([^{3}H]-OMDA, 51%), and [^{3}H]-normetanephrine ([^{3}H]-NMN, 2%). This outflow was not altered by McN-A-343 (10^{-4} M). The time course of the effect of McN-A-343 on the pattern of [^{3}H]-NA and its [^{3}H]-metabolites evoked by field stimulation was examined. The overflow from untreated artery *during* stimulation consisted of [^{3}H]-NA (28%), [^{3}H]-DOPEG (10%), [^{3}H]-DOMA (4%), [^{3}H]-OMDA (52%), and [^{3}H]-NMN (6%). Initially McN-A-343 only decreased [^{3}H]-DOPEG. Subsequently the amount of [^{3}H]-NA and [^{3}H]-NMN was also reduced with a corresponding rise in [^{3}H]-DOMA and [^{3}H]-OMDA.

The ability of various drugs to influence the biphasic response (potentiation of stimulation-evoked [^{3}H]-overflow followed by inhibition) caused by a high concentration (10^{-4} M) of McN-A-343 was studied. Prior addition of either cocaine (3×10^{-5} M), atropine (3×10^{-7} M), methylatropine (10^{-5} M), hexamethonium (3×10^{-5} M) or the prostaglandin-synthetase inhibitor, suprofen (3×10^{-5} M) did not prevent the initial potentiation induced by McN-A-343. In the case of cocaine, the enhancement was continuously maintained. The other drugs either abolished or markedly reduced the subsequent inhibition normally caused by McN-A-343. However, the enhancement was not maintained, and [^{3}H]-overflow either returned to pre-drug (control) level or just below.

The ability of McN-A-343 (10^{-6}–3×10^{-4} M), cocaine (10^{-8}–3×10^{-4} M), and desmethylimipramine to reduce the neuronal uptake of [^{3}H]-NA (10^{-8} M) by rabbit isolated aorta was examined. Aorta was treated with pargyline (5×10^{-4} M) and U-0521 (3′,4′-dihydroxy-2-methylpropiophenone; 10^{-4} M) in order to inhibit monoamine oxidase and catechol-*O*-methyltransferase, respectively. McN-A-343 was a much weaker inhibitor of [^{3}H]-NA uptake than cocaine and desmethylimipramine.

It is concluded that McN-A-343 enhances the stimulation-evoked [^{3}H]-overflow by inhibition of the neuronal membrane pump and by facilitation of transmitter release. The enhancement is not mediated by muscarinic or nicotinic receptors and is prostaglandin-independent. McN-A-343 may possibly be transported by the membrane amine pump into an intraneuronal site of inhibitory action.

Supported by the Danish Medical Research Council and P. Carl Petersen's Foundation.

Reference

ROSZKOWSKI, A.P. (1961). An unusual type of ganglionic stimulant. *J. Pharmac. exp. Ther.*, **132**, 156–170.

Catecholamine receptors in thoracic spinal cord

J.H. COOTE, S.M. FLEETWOOD-WALKER & P.R. MITCHELL

Department of Physiology, and MRC Neuropharmacology Unit, The Medical School, Birmingham B15 2TJ

The existence of bulbospinal catecholamine pathways which have a dense termination in the sympathetic intermediolateral cell column of the thoraco-lumbar cord is now well established (Dahlstrom & Fuxe, 1965; Coote & Macleod, 1974; Coote, Fleetwood-Walker & Martin, 1979). In the cat, noradrenaline, dopamine and adrenaline are found in this region, although adrenaline is present in extremely small amounts (Coote, Fleetwood-Walker & Martin, unpublished). To try to establish a transmitter role for these catecholamines in the cat thoracic spinal cord,

we performed radiolabelled ligand binding experiments to investigate the presence of specific recognition sites.

Tissue was homogenised by sonication in Tris buffer and twice-washed membrane fragments were used for filtration assays (on 20 mg tissue, or 10 mg microdissected regions).

The α-adrenoceptor binding assay was carried out essentially according to U'Prichard, Greenberg & Snyder (1977): 0.25 nM [^{3}H]-WB-4101 was used as ligand and non-specific binding determined in the presence of (−)-noradrenaline (100 μM), which gave maximal displacement in thoracic spinal cord (36% specific binding) and in cerebral cortex (58% specific binding). Preliminary Scatchard analysis of specific WB 4101 binding in whole thoracic spinal cord suggested the presence of just one type of binding site.

The β-adrenoceptor binding method was essentially that of Bylund & Snyder (1976): [^{3}H]-(−)-dihydroalprenolol (1.0 nM) was used as ligand and non-specific binding determined using (−)-isoprenaline (100 μM), which gave maximal displacement (67% specific binding) in cerebral cortex. However in cat and rat thoracic spinal cord we found negligible specific binding.

Dopamine receptor binding experiments were carried out with [^{3}H]-haloperidol (2.0 nM) as ligand, basically according to Creese, Burt & Snyder, 1975. Non-specific binding was determined with 10 μM ADTN (proposed as a DA-selective displacer of neuroleptics (Quik, Iversen, Larder & Mackay, 1978) and trial experiments on striatum gave 30% specific binding. However, we found negligible specific binding of haloperidol in spinal cord.

These results demonstrate the presence of catecholamine receptor sites in cat thoracic spinal cord which are predominantly of the α-adrenoceptor type.

Thanks to: Boehringer Ingelheim Ltd., Professor P.B. Bradley and Dr. I.L. Martin.

References

BYLUND, D.B. & SNYDER, S.H. (1976). Beta adrenergic receptor binding in membrane preparations from mammalian brain. *Mol. Pharmac.*, **12**, 568–580.

COOTE, J.H., FLEETWOOD-WALKER, S.M. & MARTIN, I.L. (1979). The origin of the catecholamine innervation of the sympathetic lateral column. Communication to the Physiological Society, Cambridge June 22–23rd.

COOTE, J.H. & MACLEOD, V.H. (1974). The influence of bulbospinal monoaminergic pathways on sympathetic nerve activity. *J. Physiol., Lond.*, **241**, 453–475.

CREESE, I., BURT, D.R. & SNYDER, S.H. (1975). Dopamine receptor binding: differentiation of agonist and antagonist states with ^{3}H-dopamine and ^{3}H-haloperidol. *Life Sci.*, **17**, 993–1002.

DAHLSTROM, A. & FUXE, K. (1965). Evidence for the existence of monoamine neurones in the central nervous system. II. Experimentally induced changes in intraneuronal amine levels of bulbospinal neuron systems. *Acta physiol. scand.*, **64**, Suppl. 247, 5–36.

QUIK, M., IVERSEN, L.L., LARDER, A. & MACKAY, A.V.P. (1978). Use of ADTN to define specific ^{3}H-spiperone binding to receptors in brain. *Nature, Lond.*, **274**, 513–514.

U'PRICHARD, D.C., GREENBERG, D.A. & SNYDER, S.H. (1977). Binding characteristics of a radiolabeled agonist and antagonist at central nervous system alpha noradrenergic receptors. *Mol. Pharmac.*, **13**, 454–473.

Table 1 Distribution of [^{3}H]-WB-4101 binding in cat thoracic spinal cord

Region	*Specific binding f mol/mg Pr.*	
Whole thoracic cord	3.02 ± 0.25 (n = 6)	
White matter W	1.48 ± 0.44	
Dorsal grey DG	3.64 ± 0.35	*n = 5
Ventral grey VG	3.10 ± 0.30	*
Intermediolateral IMG + intermediomedial grey	5.64 ± 0.38	*†
Frontal cerebral cortex	28.4 ± 1.0 (n = 6)	

* Significantly different from W level.
† Significantly different from DG and VG levels.