creased the inhibitory effect of morphine at all frequencies of stimulation ($P<0.05$: t-test).

Mice were made tolerant to morphine using a single subcutaneous injection of a sustained release preparation of morphine base (800 mg/kg) suspended in a. emulsion (Collier, Francis & Schneider, 1972). After 48 h mice were killed and their vasa removed. In these vasa the concentration inhibition curves for morphine and levorphanol (at 0.2 Hz) were moved to the right with a decreased maximum inhibitory effect compared with the effects of the same drugs in vasa from naive mice. In addition, morphine (10 μM) and levorphanol (3 μM) produced less inhibition of the twitch between 0.2 and 10 Hz than in 'naive' vasa. Lowering the calcium concentration of the Krebs solution (to 1.25 mM) increased the inhibitory effect of morphine and levorphanol at all frequencies (0.2–16 Hz) in vasa from morphine tolerant mice.

It is concluded that tolerance develops to the action of morphine in the mouse vas deferens. In morphine pretreated mice the inhibitory effect of levorphanol was also reduced showing that cross-tolerance existed between the two agonists. The development of tolerance does not prevent the modulation by calcium of the effects of opiates on neurotransmission.

We thank the Wellcome Trust for supporting DGLP.

References

COLLIER, H.O.J., FRANCIS, D.L. & SCHNEIDER, C. (1972). Modification of morphine withdrawal by drugs interacting with humoral mechanisms; some contradictions and their interpretation. *Nature (Lond.)*, **237**, 220–223.

HENDERSON, G., HUGHES, J. & KOSTERLITZ, H.W. (1972). A new example of a morphine-sensitive neuroeffector junction: adrenergic transmission in the mouse vas deferens. *Br. J. Pharmac.*, **46**, 764–766.

Effects of mianserin on noradrenaline uptake, cardiac presynaptic and vascular postsynaptic α-adrenoceptors in rats

I. CAVERO, R. GOMENI, F. LEFÈVRE-BORG & A.G. ROACH

SYNTHELABO, L.E.R.S., Dept. of Biology, Cardiovascular Unit, 58, rue de la Glacière, 75621 Paris, France

Recently, Robson, Antonaccio, Saelens & Liebman (1978) reached the conclusion that mianserin is a selective antagonist of cardiac presynaptic α-adrenoceptors since this compound did not modify noradrenaline pressor responses whilst it antagonized the clonidine-induced inhibition of the heart rate increases to short term stimulation of thoracic spinal cord. However, these authors did not study the effects of mianserin on heart rate responses to exogenous noradrenaline or spinal cord stimulation in order to assess a possible action on noradrenaline uptake.

In pithed normotensive rats in which baseline heart rate was increased by approximately 50 bts/min by sustained electrical stimulation of the thoracic spinal cord (Roach, Lefèvre & Cavero, 1978), i.v. infusion of mianserin (0.1 mg kg^{-1} min^{-1} for 10 min) or desipramine (0.01 mg kg^{-1} min^{-1} for 10 min) further increased heart rate. The latter effect was also observed when the animals were pretreated with phentolamine (0.25 mg/kg, i.v.). Furthermore, in pithed rats with 100 bts/min tachycardia the dose of clonidine producing a 50 bts/min (ED_{50}) fall in heart rate was 3.1 and 3.7 μg/kg, i.v. in control and desipramine (0.1 mg/kg, i.v.) pretreated animals, respectively. However, the control ED_{50} doses of clonidine were increased approximately 3 and 9 times after mianserin (3.0 mg/kg, i.v.) and phentolamine (0.25 mg/kg, i.v.), respectively. In the same experiments the dose of clonidine producing 50 mmHg increases in diastolic carotid blood pressure was not altered by desipramine, but it was significantly increased by mianserin and phentolamine. In pithed rats, the control heart rate frequency-response curves to short period spinal cord stimulation were shifted to the right by clonidine. Phentolamine, desipramine and mianserin abolished this effect.

Mianserin potentiated heart rate increases to exogenous noradrenaline and adrenaline as well as to short term electrical stimulation of the spinal cord. However, it did not change the tachycardia to isoprenaline whilst it decreased the heart rate response to tyramine. The pressor responses to cirazoline, adrenaline and 5-hydroxytryptamine were decreased by mianserin. However, this compound did not significantly modify blood pressure increases elicited by noradrenaline or electrical stimulation of the spinal cord.

In conclusion, these findings indicate that in the doses utilized in this study mianserin interferes with noradrenaline reuptake. Additionally, this compound possesses weak vascular postsynaptic and cardiac presynaptic α-adrenoceptor blocking properties. Certain methods used to study cardiac presynaptic α-adrenoceptors (short term electrical stimulation of the thoracic spinal cord, as in the report of Robson *et al.*, 1978) appear not to distinguish between compounds interfering with noradrenaline uptake and compounds blocking presynaptic α-adrenoceptors.

References

ROACH, A.G., LEFEVRE, F. & CAVERO, I. (1978). Effects of

prazosin and phentolamine on cardiac presynaptic α-adrenoceptors in the cat, dog and rat. *Clin. Exper. Hypertension*, **1**, 87–101.

ROBSON, R.D., ANTONACCIO, M.J., SAELENS, J.K. & LIEBMAN, J. (1978). Antagonism by mianserin and classical α-adrenoceptor blocking drugs of some cardiovascular and behavioural effects of clonidine. *Eur. J. Pharmac.*, **47**, 431–442.

Metiamide – absence of presynaptic α-adrenoceptor antagonist properties in the pithed rat

J.C. DOXEY & J. EVERITT

Reckitt and Colman, Pharmaceutical Division, Dansom Lane, Hull HU8 7DS

The antagonism by metiamide of the hypotensive effects of clonidine has prompted the suggestion that the hypotensive effect is mediated in part by stimulating histamine H_2 receptors in the central nervous system of the rat, (Karppanen, Paakkari & Paakkari, 1977). The hypotensive effect of clonidine is also antagonized by α-adrenoceptor antagonists (Schmitt, Schmitt & Fénard, 1971). It has been reported recently that metiamide is an antagonist at presynaptic α-adrenoceptors in the mouse vas deferens (Griffith, Marshall & Nasmyth, 1978). The interaction of metiamide with presynaptic α-adrenoceptors has been studied in the rat using both *in vitro* and *in vivo* models.

Vasa deferentia from CFY rats were suspended in Krebs and stimulated at a frequency of 0.1 Hz as described previously (Doxey, Smith & Walker, 1977). The twitch response was inhibited by clonidine (3 ng/ml). Metiamide (1–10 μg/ml) and phentolamine (10–300 ng/ml) produced a dose related antagonism of the clonidine inhibition. These studies confirmed previous experiments carried out in the mouse vas deferens by Griffith, Marshall & Nasmyth (1978).

In the pithed rat presynaptic activity was assessed by determining the ability of metiamide to reverse the inhibitory effects of clonidine (30 μg/kg i.v.) on sympathetic outflow from cardiac nerves (Drew, 1976; Doxey & Everitt, 1977) and hypogastric nerves (Doxey & Everitt, 1977). Post synaptic antagonism was assessed by determining the inhibition of the pressor response associated with clonidine.

Cardiac acceleration was induced by stimulation of the sympathetic outflow at either 1 Hz, 10 v, 0.5 ms continuously (Drew, 1976) or 1 Hz, 10 v, 0.5 ms for 10 s every 2 min (Doxey & Everitt, 1977). Hypogastric outflow was induced using stimulus parameters of 20 v, 50 μs, 6 Hz for 3 s every 30 seconds. Metiamide in doses up to 3 mg/kg i.v. had no effect on the inhibitory action of clonidine on either cardiac or hypogastric nerves. The clonidine pressor response was also unaffected by metiamide. The reversibility of the effects of clonidine on cardiac nerves, hypogastric nerves and blood pressure was verified by injecting phentolamine (1 mg/kg i.v.) at the end of each experiment. This dose of phentolamine produced complete reversal in all experiments. It has been shown previously that the threshold dose of phentolamine required to antagonise clonidine on hypogastric nerves and cardiac nerves was 10–30 μg/kg i.v. (Drew, 1976; Doxey & Easingwood, 1978).

In conclusion metiamide, in doses up to 3 mg/kg i.v., had no antagonistic effect at peripheral pre- and postsynaptic α-adrenoceptors in the pithed rat.

References

DOXEY, J.C. & EASINGWOOD, R.E. (1978). Profiles of α-adrenoceptor antagonists in the pithed rat. *Br. J. Pharmac.*, **63**, 401–402P.

DOXEY, J.C. & EVERITT, J. (1977). Inhibitory effects of clonidine on responses to sympathetic nerve stimulation in the pithed rat. *Br. J. Pharmac.*, **61**, 559–566.

DOXEY, J.C., SMITH, C.F.C. & WALKER, J.M. (1977). Selectivity of blocking agents for pre- and postsynaptic α-adrenoceptors. *Br. J. Pharmac.*, **60**, 91–96.

DREW, G.M. (1976). Effects of α-adrenoceptor agonists and antagonists on pre- and postsynaptically located α-adrenoceptors. *Eur. J. Pharmac.*, **36**, 313–320.

GRIFFITH, O.R., MARSHALL, J. & NASMYTH, P.A. (1978). Blockade of pre-synaptic α-adrenoceptors by metiamide. *Br. J. Pharmac.* In Press.

KARPPANEN, H., PAAKKARI, I. & PAAKKARI, P. (1977). Further evidence for central histamine H_2 receptor involvement in the hypotensive effect of clonidine in the rat. *Eur. J. Pharmac.*, **42**, 299–302.

SCHMITT, H., SCHMITT, H. & FENARD, S. (1971). Evidence for an α-sympathomimetic component in the effects of catapresan on vasomotor centres: Antagonism by piperozane. *Eur. J. Pharmac.*, **14**, 98–100.

Effects of α-adrenoceptor agonists on peripherally evoked parasympathetic submaxillary salivation in the cat

G.J. GREEN, H. WILSON & M.S. YATES

Department of Pharmacology and Therapeutics, University of Liverpool, P.O. Box 147, Liverpool L69 3BX

Clonidine, a centrally acting antihypertensive drug, has been shown to diminish submaxillary salivation produced by either brainstem or peripheral parasympathetic nerve stimulation in anaesthetized cats