Dopamine inhibition of the twitch response of the mouse isolated vas deferens

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An inhibitory presynaptic dopamine receptor is present in the rat vas deferens (Tayo, 1977) but not in that of the guinea pig (Stjärne, 1975). To obtain more information on species differences, the possibility that a presynaptic dopamine receptor was present in the mouse vas deferens has been investigated.

Dopamine (0.3–100 μM) produced a dose-related inhibition of the twitch response (0.2 Hz, 2.0 ms pulse width) of the mouse isolated vas deferens (IC_{50} 4.50 ± 0.34 μM: mean ± s.e. mean). The dopamine receptor antagonists α-flupenthixol (100 nM) and pimozide (300 nM) did not alter the inhibitory effect of dopamine. However, yohimbine (10 nM), a selective presynaptic α-adrenoceptor antagonist in the mouse vas deferens (Marshall, Nasmyth, Nicholl & Shepperson, 1978) shifted the dopamine inhibition curve to the right.

The inhibition produced by dopamine (10 μM) was inversely proportional to the frequency of stimulation, falling from over 80% at 0.2 Hz to less than 20% at 16 Hz. This pattern is similar to that of presynaptic α-adrenoceptor agonists e.g. clonidine (Marshall *et al.*, 1978).

The effect of dopamine upon the overflow of [^{3}H]-noradrenaline was investigated in vasa preloaded with [7-^{3}H]-(−)-noradrenaline (sp. act. 7.5 Ci/mmol). [^{3}H]-noradrenaline released into the Krebs solution was separated from its [^{3}H]-metabolites (Graefe, Stefano & Langer, 1973). The control fractional noradrenaline release after stimulation (1.0 Hz, 2.0 ms, 120 s) was $1.22 \pm 0.06 \times 10^{-3}$ and this increased ($P<0.01$; t-test) to $7.19 \pm 0.80 \times 10^{-3}$ in the presence of dopamine (30 μM). Yohimbine (100 nM) did not alter the dopamine fractional noradrenaline release. The increased overflow of [^{3}H]-noradrenaline produced by dopamine was unaffected by halving the calcium concentration of the Krebs solution (to 1.25 mM) and was of similar magnitude in the absence of stimulation ($5.53 \pm 0.67 \times 10^{-3}$). In the presence of cocaine (40 μM), dopamine (30 μM) still increased the stimulated fractional release of noradrenaline ($P<0.05$) and it remained unaffected by yohimbine (100 nM).

It is concluded that the dopamine inhibition of the twitch response of the mouse vas deferens is mediated by presynaptic α-adrenoceptors and not via dopamine receptors. The dopamine inhibition may be either a direct action and/or indirect via displaced noradrenaline. The displacement of noradrenaline by dopamine was independent of stimulation and calcium ion concentration. These effects are similar to those of tyramine but unlike this drug the action of dopamine was not blocked by cocaine.

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Opiate tolerance and cross-tolerance in the mouse vas deferens

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Morphine inhibits motor transmission in the mouse vas deferens (Henderson, Hughes & Kosterlitz, 1972). This effect is antagonized by low concentrations of naloxone and therefore appears to be mediated by opiate receptors. The present work has compared the effects of two opiate agonists, morphine and levorphanol, in the isolated vas deferens from naive mice and from animals chronically exposed to morphine.

Vasa from naive mice were suspended in a magnesium-free Krebs solution. The electrically induced twitch response (0.2 Hz, 2.0 ms) was inhibited by morphine (IC_{50} 0.6 μM) and levorphanol (IC_{50} 0.03 μM). The inhibition produced by morphine (10 μM) and levorphanol (3 μM) decreased with increasing frequency of stimulation (0.2, 1.0, 5.0, 10 & 16 Hz). Halving the calcium ion concentration of the Krebs (to 1.25 mM) (or the addition of magnesium 1.2 mM) in-

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.

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

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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.

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