Br. J. Pharmac. (1979), **66** 531–535

THE EFFECT OF α-ADRENOCEPTOR ANTAGONISTS AND METIAMIDE ON CLONIDINE-INDUCED LOCOMOTOR STIMULATION IN THE INFANT RAT

Y. NOMURA & T. SEGAWA

Department of Pharmacology, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Kasumi 1-2-3, Hiroshima 734, Japan

1 Subcutaneous injections of clonidine (3.9×10^{-8} mol/kg to 3.9×10^{-6} mol/kg) produced forward locomotion and wall climbing in 7-day-old rats in a dose-dependent manner.

2 The effect was reduced significantly by a preceding intraperitoneal injection of phentolamine (7.9×10^{-6} mol/kg), phenoxybenzamine (7.4×10^{-6} mol/kg), yohimbine (1.3×10^{-6} mol/kg) or piperoxan (7.4×10^{-6} mol/kg).

3 The pA_2-values of the antagonists to the clonidine-induced locomotor hyperactivity were: 5.1 (phenoxybenzamine), 5.2 (phentolamine), 6.4 (yohimbine) and 6.0 (piperoxan).

4 Metiamide (2.5×10^{-4} mol/kg, 5.0×10^{-4} mol/kg and 1.0×10^{-3} mol/kg), a histamine H_2-receptor blocker, did not affect the clonidine-induced locomotor stimulation.

5 It is suggested that the receptors which mediate clonidine-induced locomotor stimulation could be α-adrenoceptors but not histamine H_2-receptors in the central nervous system of the infant rat.

Introduction

Clonidine-induced sedation in chicks and mice is presumed to be mediated via α-adrenoceptors in the central nervous system (Delbarre & Schmitt, 1971, 1973) as is clonidine-induced hypotension in cats and rats (Schmitt, Schmitt & Fénard, 1971, 1973; Finch, 1974). Drew, Gower & Marriott (1977) demonstrated that α-adrenoceptors which mediate clonidine-induced sedation in the adult rat more closely resemble the peripheral presynaptic α-adrenoceptors than the postsynaptic ones. On the other hand, inhibitory effects of iontophoretically applied clonidine on the firing rate of cortical cells of the rat were prevented by metiamide, a histamine H_2-receptor antagonist (Sastry & Phillis, 1977). Furthermore, several studies have suggested that hypotensive effects of clonidine in the rat may be due to stimulation of histamine receptors of the H_2-type (Karppanen, Paakkari, Paakkari, Huotari & Orma, 1976; Paakkari, Paakkari & Karppanen, 1976; Karppanen, Paakkari & Paakkari, 1977; Finch, Harvey, Hicks & Owen, 1978), although it has been demonstrated that clonidine-induced sleep in young chicks was not prevented by histamine H_2-receptor blocking agents (Vogt, 1977).

It has been shown that clonidine produces locomotor hyperactivity in the infant rat aged 7 to 14 days (Reinstein & Isaacson, 1977) and 1 to 7 days (Kellog & Lundborg, 1972; Nomura, Oki & Segawa, unpublished observations). To elucidate which type of receptors mediate the locomotor stimulating action of clonidine in the infant rat, we investigated the effects of pretreatment with phentolamine, phenoxybenzamine, yohimbine, piperoxan and metiamide on clonidine-induced locomotor stimulation.

Methods

Seven-day-old Wistar rats of either sex were used. Each rat was placed in a plastic cage (24 × 18 cm) and locomotor activity was measured with an ANIMEX activity meter (Type S, LKB Instrument). The recording began 10 min after the subcutaneous injection of clonidine (3.9×10^{-8} mol/kg, 2.0×10^{-7} mol/kg, 7.8×10^{-7} mol/kg or 3.9×10^{-6} mol/kg). Locomotor activity was measured for 10 min every 20 min for a period of 90 min and expressed as counts/50 min.

In order to examine the interactions between clonidine and antagonists, all animals were injected subcutaneously (0.1 ml/10 g of body weight) with clonidine (7.8×10^{-7} mol/kg) at the following intervals after an intraperitoneal injection of the various antagonists: phentolamine (30 min); phenoxybenzamine (120 min); yohimbine (60 min); piperoxan (30 min); metiamide (20 min). These intervals were chosen after considering both the onset and the duration of action of the

0007-1188/79/080531-05 $01.00

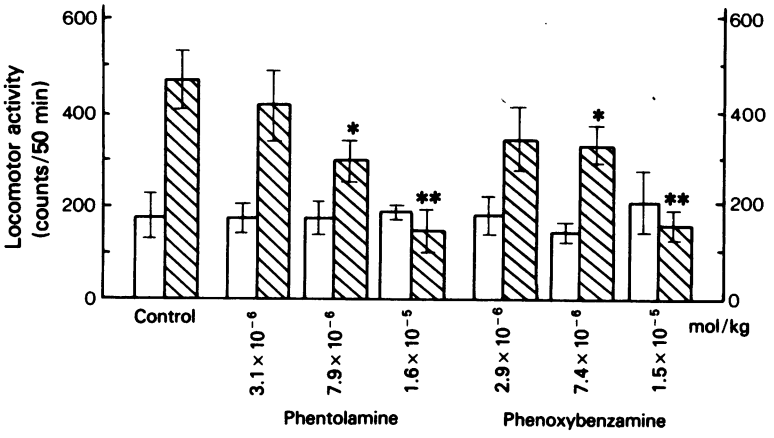

Figure 1 The effects of phentolamine and phenoxybenzamine on clonidine-induced locomotor stimulation in 7-day-old rats. Animals were injected subcutaneously with clonidine (7.8×10^{-7} mol/kg) at the following intervals after intraperitoneal injections of antagonists: phentolamine (60 min); phenoxybenzamine (120 min). The results are the mean effects on locomotor activity (counts/50 min); vertical bars show s.e. mean. Hatched columns are clonidine-treated groups and open columns are saline-treated groups. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. clonidine alone.

antagonists. Clonidine, phentolamine, phenoxybenzamine, piperoxan and yohimbine were dissolved in 0.9% w/v NaCl solution (saline). Metiamide (1.0×10^{-3} mol) was dissolved in 0.1 ml N HCl, 0.02 ml N NaOH added, and the mixture made up with water to 1 ml (pH of this solution was approximately 6). Preliminary experiments showed that the metiamide vehicle did not affect spontaneous activity or the clonidine-induced locomotor stimulation in the infant rat.

The pA_2-values of the antagonists were calculated from the shift of the dose-response curve of clonidine in the presence of the antagonists according to the equation $pA_2 = pA_x + \log(x - 1)$ proposed by Schild (1949). Student's *t* test was used for statistical analysis.

Clonidine hydrochloride was generously provided by C. H. Boeringer Zohn; phentolamine hydrochloride by Ciba–Geigy Co. Ltd.; metiamide by Smith Kline & Fujisawa K. K. Piperoxan hydrochloride was kindly donated by Dr R.D. Robson of the Pharmaceutical Division of the Ciba–Geigy Corporation. Phenoxybenzamine hydrochloride was purchased from Tokyo Kasei Ind. Co. Ltd. and yohimbine hydrochloride from Nakarai Chemicals Ltd.

Results

Clonidine-induced locomotor stimulation

Clonidine in doses ranging from 3.9×10^{-8} mol/kg to 3.9×10^{-6} mol/kg elicited forward locomotion, paddling and wall climbing in the 7-day-old rat. Locomotor activity of the rat treated with clonidine increased in a dose-dependent manner (Table 1) and the stimulation lasted for approximately 3 h.

α-Adrenoceptor antagonists on clonidine-induced locomotor stimulation

Phentolamine (1.6×10^{-5} mol/kg) did not produce loss of the righting reflex but completely blocked the clonidine- (7.8×10^{-7} mol/kg) induced forward locomotion, paddling, wall climbing and consequently the locomotor stimulation. The inhibitory effect of phentolamine on clonidine-induced hyperactivity was dose-dependent in the range 3.1×10^{-6} mol/kg to 1.6×10^{-5} mol/kg (Figure 1). Phenoxybenzamine produced loss of the righting reflex at

Table 1 Clonidine-induced locomotor stimulation in 7 day old rats

Treatment	*Locomotor activity (counts/50 min)*
Saline	94 ± 26 (17)
Clonidine 3.9×10^{-8} mol/kg	187 ± 68 (8)*
Clonidine 2.0×10^{-7} mol/kg	337 ± 57 (7)**
Clonidine 7.8×10^{-7} mol/kg	470 ± 65 (18)**
Clonidine 3.9×10^{-6} mol/kg	602 ± 173 (8)**

The results are expressed as the mean ± s.e. The number of experiments is shown in parentheses. $^{*}P < 0.05$, $^{**}P < 0.01$ when compared with the control.

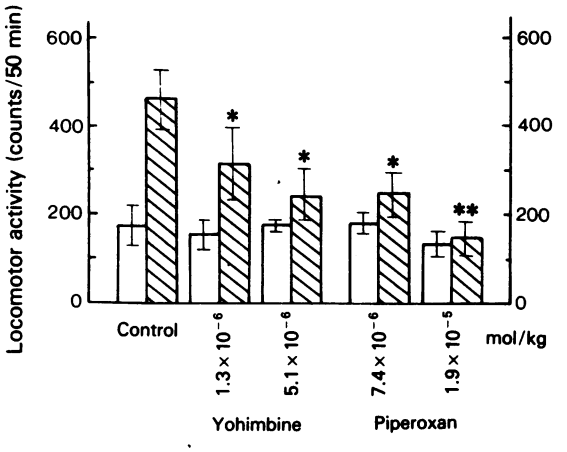

Figure 2 The effects of yohimbine and piperoxan on clonidine-induced locomotor stimulation in 7-day-old rats. Animals were injected subcutaneously with clonidine (7.8×10^{-7} mol/kg) at the following intervals after intraperitoneal injections of antagonists: yohimbine (60 min); piperoxan (30 min). The results are the mean effects on locomotor activity (counts/50 min); vertical bars show s.e. mean. Hatched columns are clonidine-treated groups and open columns are saline-treated groups. $*P < 0.05$; $**P < 0.01$ vs. clonidine alone.

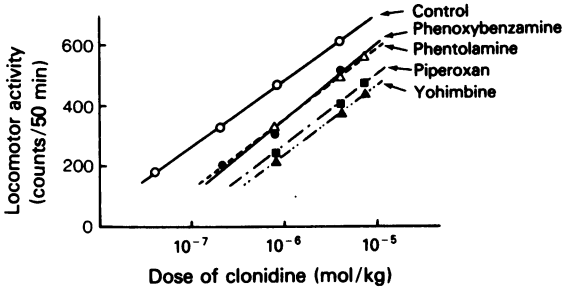

Figure 3 The effect of α-adrenoceptor antagonists on the dose-response curve for clonidine-induced locomotor stimulation. Animals were injected subcutaneously with clonidine in a dose range of 3.9×10^{-8} mol/kg to 3.9×10^{-6} mol/kg after the following intervals and doses of antagonists: phentolamine (7.9×10^{-6} mol/kg, 60 min); phenoxybenzamine (7.4×10^{-6} mol/kg, 120 min); yohimbine (5.1×10^{-6} mol/kg, 60 min); piperoxan (7.4×10^{-6} mol/kg, 30 min). The results are expressed as the mean values for locomotor activity (counts/50 min). pA_2-values were calculated from the shift of the dose-response curve of clonidine in the presence of antagonists according to the equation $pA_2 = pA_x + \log(x - 1)$ proposed by Schild (1949). (○). Control; (△). phentolamine ($pA_2 = 5.2$); (●). phenoxybenzamine ($pA_2 = 5.1$); (▲). yohimbine ($pA_2 = 6.4$); (■). piperoxan ($pA_2 = 6.0$).

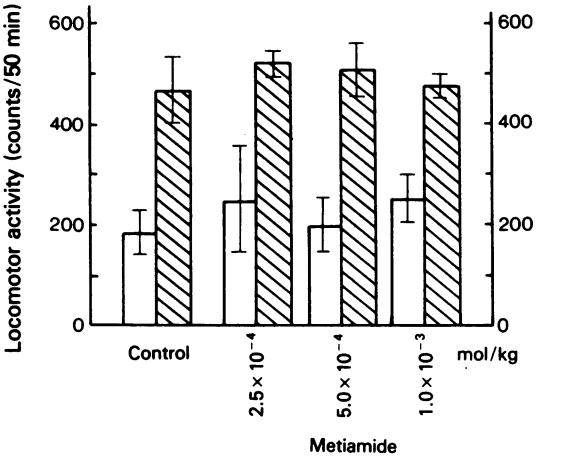

Figure 4 The effects of metiamide on clonidine-induced locomotor stimulation in the 7-day-old rats. Animals were injected subcutaneously with clonidine (7.8×10^{-7} mol/kg) 20 min after an intraperitoneal injection of metiamide. The results are the mean effects on locomotor activity (counts/50 min); vertical bars show s.e. mean. Hatched columns are clonidine-treated groups and open columns are saline-treated groups.

1.5×10^{-5} mol/kg but not at 2.9×10^{-6} mol/kg or at 7.4×10^{-6} mol/kg. Pretreatment with phenoxybenzamine (1.5×10^{-5} mol/kg) completely blocked the drug-induced forward locomotion, paddling and wall climbing. The inhibitory effect of the antagonist on the locomotor hyperactivity induced by a subcutaneous injection of clonidine (7.8×10^{-7} mol/kg) was dose-dependent (Figure 1). Yohimbine (1.3×10^{-6} mol/kg and 5.1×10^{-6} mol/kg) and piperoxan (7.4×10^{-6} mol/kg and 1.9×10^{-5} mol/kg) each produced a significant decrease ($P < 0.01$ and $P < 0.05$) in clonidine-induced locomotor stimulation (Figure 2). Righting reflex and spontaneous activity were not affected with these doses of the antagonists.

Figure 3 shows the effect of increasing concentrations of clonidine on the locomotor stimulation in the infant in combination with phentolamine (7.9×10^{-6} mol/kg), phenoxybenzamine (7.4×10^{-6} mol/kg), yohimbine (5.1×10^{-6} mol/kg) or piperoxan (7.4×10^{-6} mol/kg). From these results, pA_2-values were calculated as follows: 5.2 (phentolamine); 5.1 (phenoxybenzamine); 6.4 (yohimbine); 6.0 (piperoxan).

Metiamide on clonidine-induced locomotor stimulation

Metiamide (2.5×10^{-4} mol/kg, 5.0×10^{-4} mol/kg and 1.0×10^{-3} mol/kg) produced neither catalepsy nor loss of the righting reflex. In addition, neither

forward locomotion nor wall climbing induced by clonidine (7.8×10^{-7} mol/kg) was affected by pretreatment with metiamide at these doses. The stimulation of locomotor activity by clonidine (7.8×10^{-7} mol/kg) was not altered by an intraperitoneal injection of metiamide (Figure 4).

Discussion

Clonidine produced intense forward crawling in the 7-day-old rat, which suggests that, at this interval after birth, there is already a functional noradrenergic system that controls behaviour. From the fact that functional noradrenergic neurones are present in the cortex and brain stem but not in the diencephalon by 4 days of age, Kellog & Wennerström (1974) have suggested that the locomotor hyperactivity induced by clonidine could be initiated through these regions in the infant rat. The present results demonstrate that the systemic administration of phentolamine, phenoxybenzamine, yohimbine or piperoxan significantly reduce the clonidine-induced hyperactivity, indicating that the effect is mediated by central α-adrenoceptors in the infant rat.

Since phentolamine and phenoxybenzamine have been found to be more active on postsynaptic α-adrenoceptors in the isolated cat spleen (Cubeddu, Barnes, Langer & Weiner, 1974), significant inhibition of the locomotor activity by these antagonists in the present experiments, suggests the interaction of clonidine with postsynaptic α-adrenoceptors in the infant rat. However, yohimbine and piperoxan had higher pA_2-values than phentolamine and phenoxybenzamine against the clonidine-induced hyperactivity. Furthermore, yohimbine and piperoxan possess a higher affinity for presynaptic α-adrenoceptors than for postsynaptic ones and inhibit the effect of clonidine on presynaptic α-adrenoceptors (Cubbedu *et al.*, 1974; Starke, Borowski & Endo, 1975; Andén, Grabowska & Strömbom, 1976; Lassen, 1978; Robson, Antonaccio, Saelens & Liebman, 1978). Both phentolamine and phenoxybenzamine in concentrations of 10 and 15 μM respectively can block presynaptic α-adrenoceptors in the mouse vas deferens (Marshall, Nasmyth & Shepperson, 1977). Since higher doses of these antagonists than of yohimbine or piperoxan were required to produce comparable inhibition of locomotor activity induced by clonidine, it is possible that all the drugs were acting via α_2-adrenoceptors (presynaptic type). However, because of the variables in the *in vivo* experiment, which cannot be eliminated, the actual concentrations of the α-adrenoceptor antagonists achieved in the CNS is unknown and an action on α_1-adrenoceptors (postsynaptic type) cannot be eliminated.

It is known that metiamide is a strongly ionized compound that does not easily penetrate into the brain (Cross, 1973). Since the blood-brain barrier derived from astroglial cells and endothelial cells, is not fully functional at birth and reaches maturity at around 10 days to 2 weeks after birth in the rat, metiamide probably could pass the barrier in the 7-day-old rat. However, pretreatment with metiamide (2.5×10^{-4} mol/kg to 1.0×10^{-3} mol/kg, i.p.) did not affect the clonidine-induced hyperactivity (Figure 4). The present results are consistent with those of Vogt (1977) who found that metiamide had no effect on clonidine-induced sleep in newly-hatched chicks. It seems probable that histamine receptors of the H_2-type are not involved in clonidine-induced hyperactivity. It is also possible that H_2-receptors are not functional by the 7th day after birth. Thus, the locomotor effects of clonidine in the present experiments are mediated via α-adrenoceptors, rather than H_2-type histamine receptors.

The authors are grateful to Dr R.D. Robson, Ciba-Geigy Corporation, U.S.A., for his generous supply of piperoxan.

References

ANDÉN, N.-E., GRABOWSKA, M. & STRÖMBOM, U. (1976). Different alpha-adrenoceptors in the central nervous system mediating biochemical and functional effects of clonidine and receptor blocking agents. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **292**, 43–52.

CROSS, S.A.M. (1973). Distribution of histamine, burimamide and metiamide and their interactions as shown by autography. In *Proceedings of the International Symposium on Histamine H_2-Receptor Antagonist*. ed. Wood, C.J. & Simkins, M.A. pp. 73–86. Welwyn Garden City: Smith, Kline & French Labs Ltd.

CUBEDDU, L., BARNES, F.M., LANGER, S.Z. & WEINER, N. (1974). Release of norepinephrine and dopamine-β-hydroxylase by nerve stimulation—1. Role of neuronal and extraneuronal uptake of α-presynaptic receptors. *J. Pharmac. exp. Ther.*, **190**, 431–450.

DELBARRE, B. & SCHMITT, H. (1971). Sedative effects of α-sympathomimetic drugs and their antagonism by adrenergic and cholinergic blocking drugs. *Eur. J. Pharmac.*, **13**, 356–363.

DELBARRE, B. & SCHMITT, H. (1973). A further attempt to characterize sedative receptors activated by clonidine in chickens and mice. *Eur. J. Pharmac.*, **22**, 355–359.

DREW, G.M., GOWER, A.J. & MARRIOTT, A.S. (1977). Pharmacological characterization of α-adrenoceptors which

mediate clonidine-induced sedation. *Br. J. Pharmac.*, **61**, 468P.

FINCH, L. (1974). The cardiovascular effects of intraventricular clonidine and BAY 1470 in conscious hypertensive cats. *Br. J. Pharmac.*, **52**, 333–338.

FINCH, L., HARVEY, C.A., HICKS, P.E. & OWEN, D.A.A. (1978). Clonidine-induced hypotension: further evidence for a central interaction with histamine H_2-receptor antagonists in the rat. *Neuropharmac.*, **17**, 307–313.

KARPPANEN, H., PAAKKARI, I., PAAKKARI, P., HUOTARI, R. & ORMA, A.-L. (1976). Possible involvement of central histamine H_2-receptors in the hypotensive effect of clonidine. *Nature, Lond.*, **259**, 587–588.

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.

KELLOG, C. & LUNDBORG, P. (1972). Ontogenic variations in responses to L-DOPA and monoamine receptor-stimulating agents. *Psychpharmacologia*, **23**, 187–200.

KELLOGG, C. & WENNERSTRÖM, G. (1974). An ontogenic study on the effect of catecholamine receptor stimulating agents on the turnover of noradrenaline and dopamine in the brain. *Brain Res.*, **79**, 451–464.

LASSEN, J.B. (1978). Piperoxane reduces the effects of clonidine on aggression in mice and on noradrenaline dependent hyperactivity in rats. *Eur. J. Pharmac.*, **47**, 45–49.

MARSHALL, I., NASMYTH, P.A. & SHEPPERSON, N.B. (1977). The relationship between presynaptic α-adrenoceptors stimulation frequency and calcium. *Br. J. Pharmac.*, **61**, 128P.

PAAKKARI, I., PAAKKARI, P. & KARPPANEN, H. (1976). Antagonism of the central hypotensive effect of clonidine by the histamine H_2-receptor blocking agent, metiamide. *Acta physiol. scand., Suppl.* **440**, 105.

REINSTEIN, D.K. & ISAACSON, R.L. (1977). Clonidine sensitivity in the developing rat. *Brain Res.*, **135**, 378–382.

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 behavioral effects of clonidine. *Eur. J. Pharmac.*, **47**, 431–442.

SASTRY, B.S.R. & PHILLIS, J.W. (1977). Evidence that clonidine can cativate histamine H_2-receptors in rats cerebral cortex. *Neuropharmac.*, **16**, 223–225.

SCHILD, H.O. (1949). pA_x and competitive drug antagonism. *Br. J. Pharmac. Chemother.*, **4**, 277–280.

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

SCHMITT, H., SCHMITT, H. & FÉNARD, S. (1973). Action of α-adrenergic drugs on sympathetic centres and their interactions with the central sympathoinhibitory effects of clonidine. *Arzneimittel-Forsch.*, **23**, 40–45.

STARKE, K., BOROWSKI, E. & ENDO, T. (1975). Preferential blockade of presynaptic α-adrenoceptors by yohimbine. *Eur. J. Pharmac.*, **34**, 385–388.

VOGT, M. (1977). Histamine H_2-receptors in the brain and sleep produced by clonidine. *Br. J. Pharmac.*, **61**, 441–443.

(Received September 19, 1978.
Revised January 15, 1979.)