Inability of methylphenidate or mazindol to prevent the lowering of 3,4-dihydroxyphenylacetic acid in rat brain by amphetamine

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Recently Ross (1978) reported that methylphenidate antagonized the stereotyped behaviour produced by amphetamine in reserpinized rats. His experiment was possible because, although methylphenidate and amphetamine both cause stereotyped behaviour in normal rats, reserpine prevents that effect of methylphenidate but not of amphetamine. A possible explanation that Ross (1978) suggested for his findings was that methylphenidate inhibited the postulated active uptake of amphetamine into the dopamine nerve terminals, thereby preventing the release of dopamine by amphetamine (such release is thought to be the mechanism by which stereotypy is produced by amphetamine). One way in which the effect of amphetamine on dopamine neurons can be measured biochemically is the lowering of 3,4-dihydroxyphenylacetic acid (DOPAC) (Roffler-Tarlov et al 1971). This paper addresses the question: does methylphenidate block this biochemical effect of amphetamine in the same way that it blocks the behavioural effect (stereotypy) of amphetamine?

Male Wistar rats, 130–150 g (from Harlan Industries, Cumberland, Indiana), were used. Methylphenidate hydrochloride was a gift from Ciba. (±)-Amphetamine sulphate was purchased from Chemical Procurements, (+)-amphetamine sulphate from Sigma, and (±)-*p*-chloroamphetamine hydrochloride from the Regis Chemical Company. Reserpine (Sandril) was a product of Eli Lilly and Company. After drug treatment, rats were decapitated and whole brains were quickly removed and frozen on dry ice, then stored at −15 °C before analysis. DOPAC concentration in whole brain was determined spectrofluorometrically by use of the methods of Murphy et al (1969) and Spano & Neff (1971). Mean values ±-standard errors for 5 rats are given in all cases.

Table 1 shows that the lowering of brain DOPAC by amphetamine was not prevented by methylphenidate pretreatment either in control rats or in reserpinized rats. This experiment was patterned directly after that of Ross (1978). In non-reserpinized rats, amphetamine lowered DOPAC by 39%, and this effect was not prevented by methylphenidate. In reserpinized rats, brain DOPAC was increased to more than twice that in control rats. Whereas methylphenidate slightly increased DOPAC in normal rats, it significantly decreased DOPAC concentration in reserpinized rats, as observed earlier by Braestrup (1977). Amphetamine lowered DOPAC in reserpinized rats, and this effect was not prevented by methylphenidate.

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Table 1. Lowering of brain DOPAC by amphetamine in control and methylphenidate-pretreated rats. (+)-Amphetamine sulphate (5 mg kg^{-1}, i.p.) was injected i.p. 30 min after methylphenidate (40 mg kg^{-1}, i.p.) and 1 h before the rats were killed. Some rats were treated with reserpine (5 mg kg^{-1} i.p.) 5 1/2 h before they were killed.

Treatment	Brain DOPAC, ng g^{-1} Control	Methylphenidate-pretreated
1. Non-reserpinized rats		
Saline	67 ± 2	75 ± 5
Amphetamine	41 ± 4* (−39%)	41 ± 3* (−45%)
2. Reserpinized rats		
Saline	177 ± 3	93 ± 10
Amphetamine	53 ± 2* (−70%)	38 ± 2* (−59%)

* Significant lowering compared to the corresponding saline group, $P < 0.01$.

In non-reserpinized rats, this high (40 mg kg^{-1}) dose of methylphenidate caused pronounced stereotyped hyperactivity (increased locomotion, sniffing, gnawing, backing). Amphetamine alone caused similar effects but to a lesser degree, and the combination of methylphenidate and amphetamine produced approximately additive effects. In reserpinized rats, stereotypy did not occur after methylphenidate, though a slight increase in locomotion was still present. Amphetamine caused a greater increase in stereotyped hyperactivity in reserpinized rats than it did in non-reserpinized rats. Methylphenidate blocked the stereotyped behaviour (gnawing, sniffing and searching) induced by amphetamine in reserpinized rats, though some increased locomotor activity was still present. The ability of methylphenidate to block the stereotypy induced by amphetamine is in agreement with the findings reported by Ross (1978).

Table 2 shows a second experiment done with a different inhibitor of uptake into dopamine neurons–mazindol–and with *p*-chloroamphetamine as well as amphetamine. *p*-Chloroamphetamine has been shown earlier to lower DOPAC much the same as does amphetamine (Fuller et al 1976). Mazindol falls into the methylphenidate group of drugs in that it acts synergistically with a dopamine receptor blocker to increase brain DOPAC (Fuller & Snoddy 1978) but is an even more potent inhibitor of dopamine uptake than is methylphenidate (Koe 1976). Mazindol, at a dose previously shown to be effective in enhancing the

Table 2. Lowering of brain DOPAC by amphetamine and *p*-chloroamphetamine in control and mazindol-pretreated rats. (±)-Amphetamine sulphate and (±)-*p*-chloroamphetamine hydrochloride were injected i.p. at 15 mg kg^{-1} 15 min after mazindol (15 mg kg^{-1} i.p.) and 1 h before rats were killed.

Treatment	Brain DOPAC, ng g^{-1} Control	Mazindol-pretreated
Saline	104 ± 4	138 ± 11
Amphetamine	62 ± 3* (−40%)	71 ± 7* (−49%)
p-Chloroamphetamine	44 ± 2* (−58%)	71 ± 5* (−49%)

* Significant lowering compared to the corresponding saline group, $P < 0.01$.

spiperone-induced rise in brain DOPAC (Fuller & Snoddy 1978), did not prevent the decrease in brain DOPAC caused by either amphetamine or *p*-chloroamphetamine.

If amphetamine is actively transported into the dopamine neuron via the membrane uptake pump, and if the lowering of DOPAC by amphetamine depends on its active uptake, then uptake inhibitors should prevent the lowering of DOPAC by amphetamine. However, neither mazindol nor methylphenidate, two of the most active dopamine uptake inhibitors known, prevented the lowering of DOPAC by amphetamine. The ability of methylphenidate to block the stereotypy but not the DOPAC-lowering produced by amphetamine would seem most compatible with the idea that amphetamine is not dependent on the uptake pump for entry into the dopamine neuron but that dopamine released non-exocytotically (Arnold et al 1977) by amphetamine is dependent on the membrane pump for transport out of the neuron (as suggested for noradrenaline by Paton, 1973). Methylphenidate, by preventing the transport of released dopamine into the synaptic cleft, prevented the stereotypy caused by amphetamine in reserpinized rats (our observations and those of Ross, 1978). However, neither methylphenidate nor mazindol prevented the lowering of DOPAC by amphetamine, since that may occur through inhibition by amphetamine of monoamine oxidase attack on dopamine within the dopamine neuron (Braestrup 1977; Green & El Hait 1978). As a consequence, the lowering of brain DOPAC by amphetamine was dissociated from increased stimulation of synaptic receptors by dopamine after amphetamine treatment.

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Bradykinin-induced flexor reflex of rat hind-limb for evaluating various analgesic drugs

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Bradykinin injected through a catheter, previously implanted in the right carotid artery of conscious rats, was found by Deffenu et al (1966) to result in dextrorotation of the head, flexion of the right fore-limb and occasionally squeaking. The technique was extended by Blane (1967) and Abe et al (1971) to the evaluation of analgesic drugs. But with these methods, assessment of the suppressive effect of drugs on the bradykinin-induced responses tends to be subjective, and functional impairment of the brain due to ligation of the right carotid artery may induce changes in susceptibility to analgesics. We report an improved method in which the bradykinin-induced flexor reflex of the hind-limb of the conscious rat was recorded and used as a measure of the nociceptive reaction.

Male Sprague-Dawley rats (200–300 g) under light ether anaesthesia, had a polyethylene cannula (0·6 mm o.d.) inserted retrogradely into the left femoral artery so that the tip was in the left common iliac artery just distal to the bifurcation of the abdominal aorta. Solutions injected through the cannula flowed into the contralateral (right) common iliac artery in which a normal blood flow was maintained. Another cannula inserted into the left femoral vein was used for drugs. Immediately after anaesthesia ended, the animal was suspended horizontally in a sleeve of cloth with slits through which the four limbs, tail and cannula were exposed, all but the right hind-limb and tail being

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