Accelerated Synthesis of Dopamine in the Rat Brain after Methadone

Previous studies from our laboratory have shown that D,L-methadone increases brain homovanillic acid (HVA) levels in rats[1]. In the present study the effect of D,L-methadone on the in vivo conversion of H^3-tyrosine to Dopamine (DA) was measured in the rat basal ganglia.

Materials and methods. Experiments were carried out in male, Sprague-Dawley rats (180–210 g), caged in groups of 4 at an environmental temperature of about 20 °C. Animals were killed by decapitation and the brains rapidly removed, the corpus striatum dissected as described by GLOWINSKI and IVERSEN[2], and kept frozen at −20 °C until analyzed.

DA and HVA were assayed fluorometrically[3]. Endogenous and labelled tyrosine and DA were separated and assayed as described by COSTA and GROPPETTI[4].

Results. In agreement with previous results[1], we found that D,L-methadone significantly increased the HVA content in the basal ganglia but did not modify DA levels. In addition, Table I shows that the dextro isomer was ineffective.

Since these results suggest that D,L-methadone increased DA turnover, we studied the effect of the drug on the in vivo conversion of H^3-tyrosine to DA. In Table II are reported the specific activities (SA) of tyrosine and DA 20 min after the i.v. injection of a pulse dose of H^3-tyrosine in control rats and in rats treated with D,L-methadone 1 h previously. The SA of tyrosine in the basal ganglia was approximately 20% higher in the animals treated with methadone than in control rats. However, the SA of DA in the basal ganglia of animals treated with methadone was 230% higher than in control rats.

Discussion. These results indicate that methadone increases the synthesis of DA in the basal ganglia. This effect seems to be rather specific since methadone did not influence serotonin metabolism in the rat brain stem[1]. The fact that dextro-methadone is inactive both in raising HVA and in producing analgesia[5], suggests that the 2 effects might be strictly correlated.

The mechanism by which methadone stimulates DA synthesis is under investigation. The possibility that the therapeutic effect of methadone on heroin withdrawal syndrome originates from its effect on DA receptors should be considered.

Recently TAMARKIN, GOODWIN and AXELROD[6] and BOWERS, KLEBER and DAVIS[7] observed a decreased accumulation of HVA levels following probenecid in methadone addicts. Their data suggest a decrease in DA turnover in these subjects. It would be of interest to clarify whether the difference between our data and theirs depends on interspecies variations or a different experimental situation, i.e. methadone addiction and acute administration of the drug.

Table I. Effect of D,L-methadone on HVA levels in the rat basal ganglia

Treatment mg/kg i.p.	Basal ganglia HVA (μg/g)	DA (μg/g)
None	0.23±0.02	3.12±0.10
D,L-methadone 10	0.48±0.02[a]	3.27±0.15
D-methadone 20	0.21±0.01	3.10±0.20

[a] $P < 0.01$ in respect to control values (Student's *t*-test). Each value is the average ±S.E. of at least 20 determination. Drugs were given 2 h before sacrifice.

Table II. Effect of D,L-methadone on the conversion of H^3-tyrosine to H^3-dopamine (DA) in the basal ganglia

Treatment (No. of animals)	Specific activities[a] 20 min after the i.v. injection of H^3-tyrosine Basal ganglia Tyrosine	DA
None (14)	432±18	112±10
D,L-methadone (12)	503±21	255±15

[a] cpm/μg/g. Each value is the average ±S.E. of the number of experiments reported in parenthese. Methadone was given i.p. at the dose of 10 mg/kg 90 min before the injection of H^3-tyrosine (L-tyrosine-3,5-H^3; 500 μCi/kg, 25 Ci/mmoles).

Riassunto. Nei ratti, il D,L-metadone fa aumentare significativamente i livelli di acido omovanillico e stimola il turnover della dopamina nei gangli della base. Il destro isomero è inattivo.

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Nitroglycerin on the Carotid Artery and Femoral Artery of the Dog

In 1953 it was shown that the aorta strip of the rabbit can be contracted maximally by adrenaline, even when high concentrations of nitroglycerin are present in the organ bath: this phenomenon was called the 'break through' of the catecholamines through the nitroglycerin inhibition[1,2]. We previously described a similar 'break through' for strips of the femoral artery or femoral vein of the dog, and for the perfused hindleg of the dog[3].

The present report deals with results showing that this 'break through' is not present in the carotid artery strip of the dog, but that this difference between vessels cannot be demonstrated in vivo.

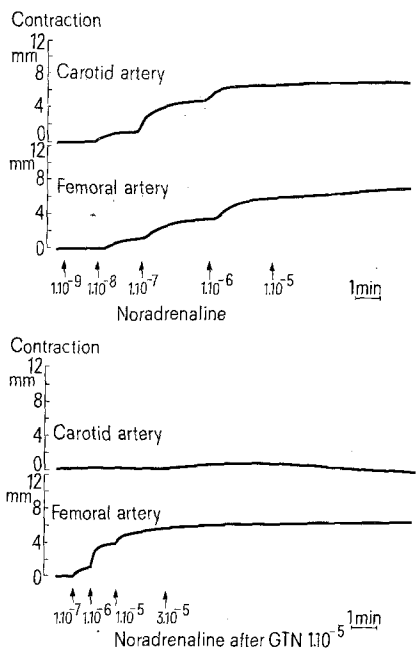
Materials and methods. Spirally cut strips of the femoral artery and the carotid artery of the dog are made to contract isotonically using a counterweight of 4 gm[1]. Increasing concentrations of the agonist, noradrenaline

(Levophed®) are added cumulatively to the bath fluid. The influence of nitroglycerin is tested by adding it to the bath fluid 5 min before the first dose of noradrenaline, and leaving it in the bath throughout the performance of the noradrenaline dose-response curve. In a series of other experiments, the strips are contracted by a supramaximal dose of noradrenaline, and, on top of this maximal contraction, increasing doses of nitroglycerin are added. Concentrations of drugs in the bath are expressed as g/ml; noradrenaline concentrations are given in terms of the base.

In Nembutal®-anesthetized dogs, carotid artery and femoral artery flows are measured by means of an electromagnetic flowmeter (Medicon), using non-cannulating probes. Systemic blood pressure is measured with a Statham-pressure transducer. Nitroglycerin is given i.v.; in some experiments noradrenaline is infused directly into the carotid or femoral artery. Denervation of the hindlegs is performed by cutting the abdominal sympathetic chains and the sciatic and femoral nerves.

Results and discussion. The contraction of a carotid artery strip and a femoral artery strip from the same dog, for increasing concentrations of noradrenaline, is shown in the Figure, both in the absence and in the presence of nitroglycerin 1.10^{-5}. After nitroglycerin, the carotid artery strip can only by contracted to a small fraction of its control value, while the femoral artery strip contracts to only slightly less than the maximal value obtained without nitroglycerin, although the effects of the lower doses of noradrenaline are inhibited, just as seen in rabbit aorta strips[1]. With 1.10^{-9} nitroglycerin, the maximal contraction of the carotid artery strip is reduced to about 50%; the same concentration of nitroglycerin has essentially no influence on the noradrenaline-induced contraction of the femoral artery strip.

When the strips are fully contracted by a supramaximal dose of noradrenaline, addition of nitroglycerin in doses as low as 1.10^{-11} relaxes carotid strips to less than 50% of their original degree of contraction; the fully contracted femoral strips show only small relaxations (around 10%), even when nitroglycerin 1.10^{-5} is added.



Isotonic contraction of carotid artery and femoral artery strips of a dog under influence of cumulatively added doses of noradrenalin. Upper part: control curves; lower part: nitroglycerin 1.10^{-5} present in the bath. Concentrations are given in g/ml.

Increasing doses of nitroglycerin intravenously lower the anesthetized dog's blood pressure in a dose-related way. Carotid artery flow increases sharply while the blood pressure falls. Femoral artery flow shows, on injection of nitroglycerin, oscillating phases of increase and decrease around the control value, with, in most dogs, a predominant decrease in flow. We previously reported that, after denervation of the hindleg, the femoral artery flow increases after nitroglycerin injection[3].

In experiments in dogs with both hindlegs denervated, on intravenous injection of 10 µg/kg of nitroglycerin, carotid artery and femoral artery flows increase while the blood pressure goes down. Similar experiments were performed while noradrenaline (0.05 to 0.2 µg/kg/min) was infused into one carotid artery or one femoral artery, the contralateral arteries serving as controls. During the infusion of noradrenaline, a dose-dependent decrease in flow in the treated arteries was obtained; femoral artery flow could be reduced to approximally zero without change in systemic blood pressure and contralateral flow; carotid artery flow could only be reduced to about 50% with doses of noradrenaline that did not yet change systemic blood pressure. Nitroglycerin, 10 µg/kg, was injected i.v. while the arteries were constricted by the different doses of noradrenaline: nitroglycerin gave smaller and smaller increases in flow as the noradrenaline-induced decrease in flow was more pronounced: this was true as well in carotid as in femoral arteries, and there was no suggestion that, for a given degree of noradrenaline-induced constriction, nitroglycerin was able to dilate the carotid artery more than the femoral artery.

Using our experimental set-up, we could thus not demonstrate in vivo the in vitro finding that nitroglycerin is a more potent antagonist for noradrenaline in the carotid artery than in the femoral artery, at least if we eliminate the influence of sympathetic reflexes that in vivo modify the responses of the vessels towards nitroglycerin.

Because of the importance of the effects of nitroglycerin on peripheral vessels[4,5], we are at present further investigating this difference in behaviour of carotid and femoral arteries in vitro (present also in the cat, but not in the rabbit, unpublished results) and its possible meaning[6].

Résumé. In vitro, la nitroglycérine antagonise aisément la contraction de l'artère carotidienne du chien provoquée par la noradrénaline, ce qui n'est pas le cas pour l'artère fémorale. In vivo cependant les différences de comportement de ces deux artères envers la nitroglycérine ne semblent pas être liées à des propriétés intrinsèques des vaisseaux, mais bien à des reflexes sympathiques.

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