Time Course of Brain MHPG-SO₄ Level Following Stimulation of Pre- and Post-Synaptic α-Adrenoceptors by Clonidine

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The concentration of 3-methoxy-4-hydroxyphenylethylene glycol sulfate (MHPG-SO₄), one of the major metabolites of noradrenaline (NA) in rat brain, has been proposed as an index of cerebral NA turnover. Manipulations which increase or decrease the rate of transmitter utilization in the CNS have caused parallel changes in brain levels of the metabolite, measured either by the sulfate conjugate or by total MHPG (free+ conjugate) (1, 2). Since conversion of NA to MHPG-SO₄ is a rapid process, acceleration of NA release results in an elevation of the metabolite level within a short time period. Korf et al. (3) reported that 7.5–15 min of stimulation of the locus coeruleus caused an increase of 40–70% of MHPG-SO₄ in the cerebral cortex, which was also associated with a corresponding decrease of endogenous NA (4). In our laboratory, activation of the central NA system by the use of stressful procedures elicited both MHPG-SO₄ elevations and NA reductions in several brain regions of rats within 15–30 min (5, 6). The decreases of the metabolite level due to lowered NA release, however, have been demonstrated hours or days after administrations of clonidine (2), reserpine, α-methyl-p-tyrosine and FLA-63 (7) and after destruction of the locus coeruleus (3). In these reports, whether the decrease in MHPG-SO₄ already begins within 120 min was not studied.

It is well described that clonidine at a low dose directly inhibits NA release in the CNS via stimulation of presynaptic α-adrenoceptors, and the drug at a high dose inhibits NA release as a consequence of negative feedback following stimulation of postsynaptic α-adrenoceptors. Furthermore, these inhibitions of NA release seem to occur rapidly since the central effects of clonidine are evident in symptoms such as inhibition of the firing rate of cells in the locus coeruleus (8) or lowering blood pressure (9), both of which appeared within a few minutes after administration. Accordingly, we have examined the time course for changes in the endogenous levels of MHPG-SO₄ and NA following injections of low and high doses of clonidine and evaluated whether these changes relate to specific regions of the brain.

Male Wistar rats (220–260 g) received clonidine hydrochloride (50 and 300 µg/kg, i.p., Boehringer) or saline (3 ml/kg, i.p.) and were sacrificed 15–120 min after injections by decapitation. Four brain regions dissected by the method of Gispen et al. (10) and the residual brain (without the cerebellum and olfactory bulbs) were frozen on dry ice and stored at −45°C until assayed. MHPG-SO₄ and NA were determined simultaneously according to a fluorometric technique developed by the authors (11).

As shown in Fig. 1A, no alteration in brain levels of MHPG-SO₄ was found 15 min after injection of 50 µg/kg clonidine. During this time period, however, the drug at 300 µg/kg caused a significant reduction of MHPG-SO₄ in the cerebral cortex (89.5% of the control value) and tended to reduce the conjugate in the pons+medulla oblongata (92.6%) as well as in the residual brain (92.6%). After 30 min, both 50 and 300 µg/kg of clonidine reduced the metabolite levels in the residual brain (87.2% and 90.4%) and 300 µg/kg of the agent also did so in the...
pons + medulla oblongata (85.6%), while slight decreases were observed in the hypothalamus (91.6%) and cerebral cortex (91.1%) by the low and high dose of clonidine, respectively. From 60 min to 120 min, the metabolite levels were significantly decreased (63-82%) with both doses of clonidine, except in the basal ganglia following the low dose administration. In contrast, NA contents in most brain regions were gradually elevated (110.5–124.5% at 120 min) following injections of clonidine, presumably by accumulation of intraneuronal storage of the transmitter (Fig. 1B). NA content in the basal ganglia did not change at any time.

The present study indicates that 10% decreases (relative to control values) in MHPG-SO₄ could be detected in specific brain regions 15 min after clonidine at 300 μg/kg and 30 min after clonidine in 50 and 300 μg/kg, although Braestrup and Nielsen (2) reported a decrease of total MHPG in the whole brain of rats 60 min after clonidine at 0.5 mg/kg, s.c. In all brain regions, MHPG-SO₄ was apparently reduced 60-120 min after clonidine injection. The accumulation of NA induced by clonidine was found after 30 min in two brain regions and became evident in most brain regions after 120 min. Overall, the decrease in NA release in the CNS is reflected earlier and more markedly by MHPG-SO₄ than by NA. The regions examined in the present study showed quite different steady state levels of MHPG-SO₄ and NA and different rates of MHPG-SO₄ production (12); however, the degree of metabolite reduction and amine elevation caused by clonidine was similar in these regions. The basal ganglia, the most dopamine-rich regions, showed relatively few changes.

Based on the present data, even if the release of NA in the CNS is rapidly inhibited,
the reduction of MHPG-\(\text{SO}_4\) levels in various brain regions may appear 30 min or longer following such inhibition. The gradual onset of MHPG-\(\text{SO}_4\) reduction might be partly explained by the low conversion rate of NA to the conjugate or total MHPG (20–35%) and by a slow elimination rate of the metabolite in the normal rat brain (decline of the metabolite 60 min after pargyline corresponds to 14–35% of the steady state values) (1, 13).

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