i.p.) 30 min before L-α-methyldopa (400 mg/kg, i.p., 6 h, n = 3) did not significantly change the level of α-methyldopamine (0·54 ± 0·086 μg/g in the brain, 0·11 ± 0·024 μg/g in the spinal cord) but markedly reduced that of α-methylnoradrenaline (0·06 ± 0·090 μg/g in the brain, 0·01 ± 0·001 μg/g in the spinal cord).

From this functional and chemical evidence it appears that there is a correlation in time of the functional effects of *m*-tyrosine and α-methyldopa and the peak accumulation of *m*-tyramine and α-methylnoradrenaline, respectively. There was a much greater and faster accumulation of *m*-tyramine than of the other amines which may be of importance for the dopamine receptor stimulation. All the β-hydroxylated amines reached about the same peak concentrations but stimulation of the noradrenaline receptors was only seen after injection of α-methyldopa.

In conclusion, treatment with *m*-tyrosine and α-methyldopa caused a stimulation of central dopamine and noradrenaline receptors, respectively, whereas no effect on these receptors was observed after treatment with α-methyl-*m*-tyrosine.

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Department of Pharmacology,
University of Göteborg,
Sweden.

NILS-ERIK ANDÉN
SHERREL G. BUTCHER
JÖRGEN ENGEL

April 8, 1970

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Effects of chronic morphine administration on the catecholamine depletion induced by reserpine

Morphine interferes with the depletion of brain noradrenaline seen after reserpine in acute (Freedman, Fram & Giarman, 1961) and chronic experiments (Gunne, 1963). After chronic administration of morphine there was only a 38% reduction of brain noradrenaline 20 h after an injection of reserpine compared with a 93% reduction in control animals without morphine.

To establish the cellular localization of these effects, especially those of the various

catecholamine terminals and cell body systems in the central nervous system (Fuxe, 1965), a histochemical catecholamine analysis (see Falck, Hillarp & others, 1962) has also been made.

Male white Sprague-Dawley rats, 250 g, had morphine HCl intraperitoneally in increasing doses up to 180 mg/kg twice daily for three weeks (for details, see Gunne, 1963). A single injection of reserpine (5 mg/kg, i.p.) was then given 4 h after the morning dose of morphine. The animals were divided into 3 groups. (i) Control group, given reserpine and killed at the same time intervals as the morphine-treated rats (4, 12, 20, 28, 48, 72 h after reserpine). (ii) Morphine-tolerant group, given morphine (180 mg/kg, every 8 h) after the reserpine. (iii) Withdrawal group not given morphine after the reserpine.

Brain and adrenal catecholamine levels were measured (Euler & Lishajko, 1961; Carlsson & Lindqvist, 1962). Animals (2 or 3) were taken for histochemistry from each experimental group. The tel- and diencephalon, mesencephalon, pons and the medulla oblongata were first reacted with formaldehyde gas, generated from paraformaldehyde stored at 70% relative humidity; and then reacted with formaldehyde gas generated from paraformaldehyde stored at 90% relative humidity (see Dahlström & Fuxe, 1964; Fuxe & Jonsson, 1967).

In the controls there was a rapid and profound depletion of noradrenaline to 11% of the starting concentration and this depletion lasted longer than in the morphine-treated groups (Fig. 1A). At 72 h after the reserpine injection the noradrenaline concentration was still 26% of normal in the controls. Continuous administration of morphine reduced the degree of depletion. The noradrenaline was 59% at 12 h after reserpine and 48% at 48 and 72 h. In the withdrawal experiment, the noradrenaline concentrations were also higher than in the controls, the mean varying between 45% at 28 h and 75% at 48 h after reserpine.

A corresponding pattern was obtained in brain dopamine (Fig. 1B), a depletion occurring in the controls during the first 28 h with tissue concentrations down to 0·5% of initial values and only an incomplete recovery at 72 h (40%). In the morphine-tolerant animals the depletion was only moderate, the tissue concentrations remaining between 43 and 75% of the starting value. The dopamine concentrations in the withdrawal group returned to normal sooner than did noradrenaline, and in both instances the 48 h amine level differed from the morphine-tolerant rats ($P<0{\cdot}05$).

The histochemical studies showed a catecholamine depletion after reserpine within cell bodies and terminals only in the control rats. At 20–28 h after reserpine, a recovery of fluorescence was seen in all catecholamine cell bodies and at the end of the experiment a clearcut recovery of fluorescence had occurred in the catecholamine nerve terminals (see Dahlström & Fuxe, 1964). In the morphine-treated animals, on the other hand, there was no noticeable reduction of fluorescence within the noradrenergic or dopaminergic neuron cell bodies. Only in the nerve terminals was there a moderately reduced degree of fluorescence.

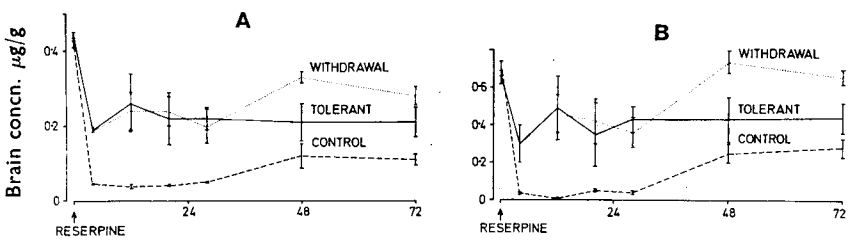

FIG. 1. The effects of a single dose (5 mg/kg, i.p.) of reserpine on whole brain concentrations of A, noradrenaline B, dopamine, at various time intervals after reserpine injection. Broken line = control group; solid line = morphine tolerant group; dotted line = withdrawal group.

The observation (Gunne, 1963) that the catecholamine-depleting action of reserpine in the brain is partially blocked by chronic morphine administration was confirmed. Furthermore, this antagonism of reserpine lasted the three day experiment, even when morphine was withheld. In the latter case the brain dopamine and noradrenaline nerve terminals had reached normal or nearly normal values within 48 h after reserpine at a time when the amine values in the controls were still low. The mechanism of this antagonism between morphine and reserpine is unknown. It has been shown, however, that the reserpine-induced catecholamine depletion can be partially reversed by pretreatment with other substances besides morphine, such as *m*-tyrosine and tetrabenazine (Quinn, Shore & Brodie, 1959; Carlsson & Lindqvist, 1967). In these cases the protection is usually believed to be the result of a competition for the uptake-storage mechanism by reserpine and the drug used for pretreatment. A similar mechanism may exist with morphine.

In the morphine withdrawal group there was a large replenishment of dopamine and noradrenaline stores 48 and 72 h after resperine. In the morphine tolerant group, the brain catecholamine remained at about 50% during the 3 day experiment, probably due to an iterated morphine-induced liberation of catecholamine (Gunne, Jonsson & Fuxe, 1969).

There was no evidence of a release of brain catecholamine from withdrawal stress in these rats. Such a depletion as a result of morphine withdrawal has been reported in dogs, but is an irregular finding in rats (Gunne, 1963). The reserpine syndrome was less severe in the morphine tolerant rats. There was less reduction in exploratory behaviour, less pronounced hunched back posture and less decrease in response to various stimuli than in the reserpine-treated control rats. It seems likely that the rapid recovery from the effects of reserpine on gross behaviour in the morphine-treated rats seen in this study is related to the incomplete depletion of brain dopamine and noradrenaline nerve terminals.

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Psychiatric Research Center,
Ulleråker Hospital,
S–750 17 *Uppsala, Sweden.*
L.-M. GUNNE
J. JONSSON

Department of Histology,
Karolinska Institutet,
S–104 01 *Stockholm, Sweden.*
K. FUXE

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