

LETTERS TO THE EDITOR

Preliminary observations on the uptake of catecholamines in synaptosomes of opiate-treated animals

The acute administration of morphine is well known to result in changes in brain catecholamine metabolism, producing a decrease in the level of noradrenaline in whole brain (Quinn & Brodie, 1961) and in hypothalamus (Moore, McCarthy & Bovison, 1965). Clouet & Rather (1970) demonstrated that morphine increases the *in vivo* biosynthesis of catecholamines from [¹⁴C]tyrosine. Smith, Villarreal & Bednarczyh (1970) reported a morphine-induced increase in the *in vivo* biosynthesis of dopamine and noradrenaline from [¹⁴C]tyrosine which was antagonized by naloxone.

Uptake of [³H]dihydromorphine by rat brain has been demonstrated *in vitro* by synaptosomes. This uptake was inhibited by narcotic antagonists (Hug & Oka, 1971). When adult rat brain homogenates are incubated with [³H]noradrenaline, most of the radioactivity is localized in the synaptosomal fraction (Coyle & Snyder, 1969).

A recent study by Ciofalo (1972) has demonstrated an inhibition of the *in vitro* synaptosomal uptake of noradrenaline by the narcotic drugs morphine, methadone, thebaine, and cocaine. The present study describes the effects of *in vivo* acute narcotic drug administration on the *in vitro* uptake of noradrenaline by synaptosomes.

Adult male rats (Sprague-Dawley, 150–200 g) were injected intraperitoneally with equal volumes of either sterile U.S.P. saline, aqueous solutions of morphine HCl (50 mg kg⁻¹), or aqueous solutions of heroin HCl (10 mg kg⁻¹). Nine animals for each whole brain experiment and 18 animals for each hypothalamic experiment were decapitated at the point of maximal drug depression, i.e., 15 min post-injection for the heroin-treated animals and 1 h post-injection for the morphine-treated animals. Controls were killed 15 min and 1 h post-injection.

The brains were quickly removed and placed in five volumes of 0.32M sucrose at 4°, tris-HCl buffered to pH 7.4. For the hypothalamic experiments, dissection according to Glowinski & Iversen (1966) was carried out rapidly after decapitation. The synaptosomes were isolated from brain according to Whittaker (1965). [³H](–)-Noradrenaline (³H-NA) was obtained from Amersham-Searle Inc.

Incubation mixtures (1 ml) contained: synaptosomes (10 mg protein), whole brain or hypothalamus; Krebs II bicarbonate solution, pH 7.4 (tris-HCl, 0.05M); 1 mM phenylisopropyl hydrazine (MAO inhibitor). Incubation mixtures (8 each) from control, heroin-treated, and morphine-treated animals were pre-incubated at 37° under 7 litres of 5% carbon dioxide in oxygen for 10 min with shaking. ³H-NA (specific activity 6.6 Ci mmol⁻¹) was added to each mixture to achieve a concentration of 5 × 10⁻⁸M. The mixtures were incubated for 20 min under the stated conditions and then rapidly chilled in an ice water bath. The synaptosomes were washed twice and the pellets were solubilized and radioactivity was determined in a liquid scintillation spectrometer.

Heroin (10 mg kg⁻¹) inhibited the uptake of ³H-NA in synaptosomes prepared from the hypothalamus, whereas morphine (50 mg kg⁻¹) did not. At dosages of 10 mg kg⁻¹ of heroin, the mean uptakes were 84% (*P* < 0.01) and 75% (*P* < 0.01) of the control

Table 1. (^3H)-(-)-noradrenaline uptake by synaptosomes prepared from whole brain and hypothalamus. Krebs II bicarbonate. Geometric means.

Conditions	Control	Heroin 10 mg kg ⁻¹	Morphine 50 mg kg ⁻¹	Significant differences	Level of significance***
Hypothalamus (Krebs)	350.3*	294.6	343.8	C vs H**	$P < 0.05$ (n = 36)
Hypothalamus (Krebs)	241.8	181.3	220.0	C vs H H vs M	$P < 0.01$ $P < 0.01$ (n = 36)
Whole brain (Krebs)	336.6	260.4	293.7	C vs H C vs M H vs M	$P < 0.01$ $P < 0.05$ $P < 0.05$ (n = 36)
Whole brain (Krebs)	493.3	296.3	321.1	C vs H C vs M	$P < 0.01$ $P < 0.01$ (n = 36)

* Results expressed as counts min⁻¹ mg⁻¹ protein.

** C = control; H = heroin; M = morphine.

*** Level of significance determined by Student's *t*-test.

values in the hypothalamic experiments. Morphine at 50 mg kg⁻¹ did not result in any change in ^3H -NA uptake when compared to control values. Both heroin and morphine inhibited ^3H -NA uptake in whole brain synaptosomes. At dosages of 10 mg kg⁻¹ of heroin, the mean uptakes were 77% ($P < 0.01$) and 65% ($P < 0.01$) of the control values. At dosages of 50 mg kg⁻¹ of morphine, the mean uptakes were 87% ($P < 0.05$) and 75% ($P < 0.01$) of the control values. (Table 1).

These preliminary experiments indicate a differential inhibitory *in vivo* effect of heroin compared to morphine on the *in vitro* synaptosomal ^3H -NA uptake when the opiates are administered in equivalent pharmacological dosages.

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