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PRESYNAPTIC EFFECT OF MORPHINE AND HALOPERIDOL ON DOPAMINE SYNTHESIS

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Bai-Chuang Shyu, Yi Chiang and Wei-Kung Wang (1978) Presynaptic effect of Morphine and Haloperidol on Dopamine Synthesis. *Bull. Inst. Zool., Academia Sinica* 17(2): 125-130. Morphine and haloperidol were added into crude synaptsomal preparation with L(1-^{14}C) tyrosine. Liberated $^{14}CO_2$ were measured continuously by a respirometer to indicate the rate of dopamine biosynthesis. Morphine was found to stimulate dopamine synthesis. This stimulation could be antagonized by nalorphine. Activation of reactions catalyzed by Catecho-O-Methyltransferase and Monoamine Oxidase after binding of pre-synaptic opiate receptor was suggested for the acute action of morphine. Haloperidol was found to stimulate dopamine synthesis at low dose and inhibite dopamine synthesis at higher dose.

Morphine and other narcotic analgesic drugs has been found to increase the incorporation of ^{14}C-Tyrosine into ^{14}C-Dopamine in the corpus striatum[7,10,24] and neuroleptics also have shown to increase dopamine turnover in corpus striatum[4,18,19,23]. Recently, it is proposed that main site of morphine action may be presynaptic, where the drug might interfere directly with the metabolism of dopamine at the corresponding terminals[14,16,17]. Although the relationship between morphine administration and dopamine metabolism has been extensively studied, there is no clear picture about the morphine action. Fukui and Takagi[10] reported that morphine do not activate the enzyme tyrosine hydroxylase directly *in vitro*, and suggested that the acceleration of dopamine biosynthesis after morphine administration may be due to a feedback activation of biosynthesis. The synaptosomal effects studied in this report may therefore be related to the presynaptic receptor, for example opiate receptor, instead of the tyrosine hydroxylase itself.

There is evidence suggesting that haloperidol is dopaminergic receptor antagonists[1,3] and is structually related to dopamine[12]. It will bind the postsynaptic dopamine receptor with high affinity and a compensatory activation of dopamine biosynthesis has been suggested[6]. In recent report, Gallager *et al.*[11] suggested that chronic haloperidol treatment produced a significant increase in the sensitivity of the presynaptic dopamine containing cells to dopamine.

The purpose of present study was to investigate the effects of morphine and haloperidol upon dopamine biosynthesis with our newly designed respirometer to prove its direct effect on presynaptic dopamine neuron.[25] Detailed dose-response relationships were studied. The ability of nalorphine to antagonize morphine induced increase in dopamine synthesis was also assessed.

MATERIALS AND METHODS

Male Sprauge-Dawley Rats (Weight 180 g-230 g) raised in our laboratory were sacrificed by decapitation. Brain were removed and dissected on ice. Corpora striata (containing caudate nucleus and a portion of putamen) were removed and homogenized in 10 volume of 0.32 M sucrose by teflon pestle tissue homogenizer with 0.25 mm clearance (from Arthur H. Thomas Co., Piladel-

phia). Homogenates were centrifuged (0°~4°C) at 1,000 g for 15 minutes to sediment nuclei and debris. Several 50 μl portions of the supernatant (equivalent to 5 mg wet tissue) containing synaptosomes and other cellular components were added to test tubes each containing 150 μl of physiological medium which contained 125 mM NaCl, 1.48 mM $CaCl_2$, 4.8 mM KCl, 2.5 mM $MgSO_4$, 22 mM NaH_2PO_4, 10 mM $NaHCO_3$ and 16 mM glucose (chemicals are from Sigma) and gave a final pH of 6.6 when equilibrated with 95% O_2–5% CO_2 gas. Tyrosine concentration was 5 μM (specific activity 50 m Ci/m mole from New England Nuclear Corp.). Morphine, haloperidol or nalorphine were added to the incubation medium with 10 μl 0.1 N phosphorous buffer pH=6.6 as carrier, control tube was also added with same 10 μl buffer. Liberated $^{14}CO_2$ from L(1-^{14}C) tyrosine were measured for every 10 minutes by a respirometer[25].

RESULTS

Increased Synthesis of Dopamine After Morphine

The time courses of the effects of various doses of morphine on the dopamine biosynthesis in corpus striatum of normal rat were shown in Fig. 1. A rapid increase in the dopamine metabolism was observed after administration of a dose, 1.0×10^{-3} M, of morphine (Fig. 1a). The amount of increase was 28% ±10% (mean+S. D.) of the phosphorous buffer control value. The amount of increase in dopamine metabolism at dose 1.0×10^{-4} M of morphine (Fig. 1b) was 15% ±3% (mean+S. D.) of the phosphorous buffer control value. No significant increase in the dopamine synthesis was observed when morphine lower than 10×10^{-5} M was administrated (Fig. 1c).

In rat, morphine induced a dose-dependent increase in the dopamine synthesis, the amount of stimulation increased with the increase of dose.

Antagonism by Nalorphine

To determine whether this effect of morphine is through dopamine or opiate receptor. The effect of nalorphine, partial antagonist of morphine, was examined. It had no significant

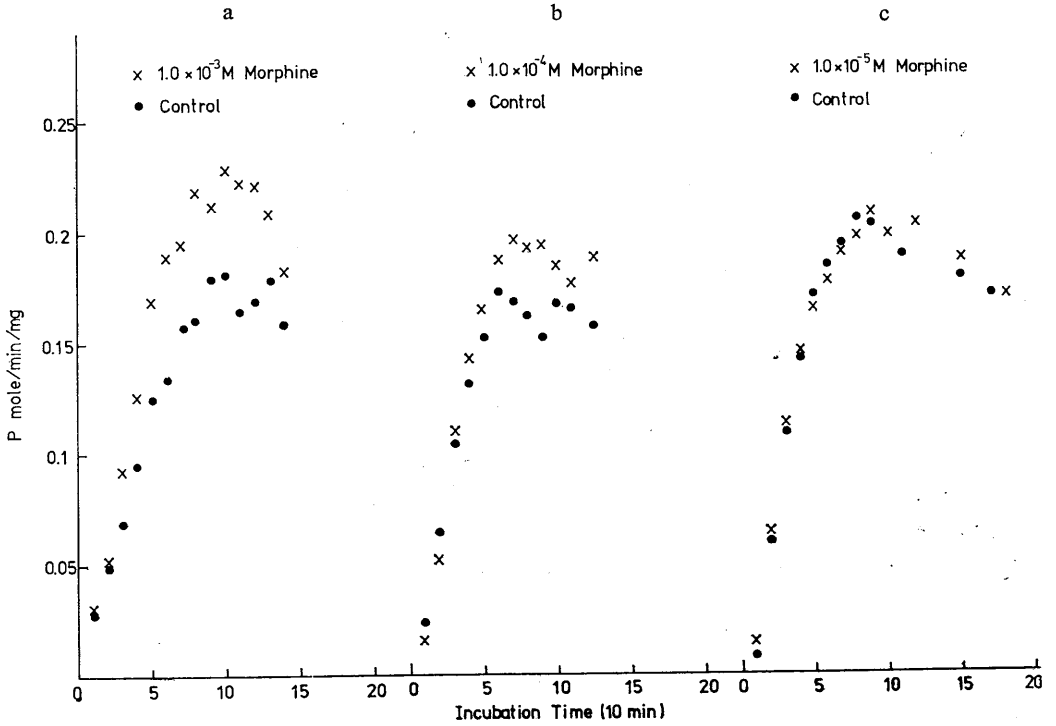

Fig. 1. Effects of Morphine on dopamine biosynthesis as function of incubation time.

effect upon the dopamine synthesis rate when nalorphine was administrated alone with doses of 7.2×10^{-4} M and 7.2×10^{-5} M. The increase in the rate of dopamine synthesis induced by morphine administration (1.0×10^{-3} M) was completely suppressed by nalorphine at dose 7.2×10^{-4} M (Fig. 2a). Even one tenth of this dose, 7.2×10^{-5} M of nalorphine antagonized morphine effect significantly (Fig. 2b). Nalorphine had no effects on inhibitory effect of dopamine synthesis induced by haloperidol.

Effect of Haloperidol on Dopamine Synthesis

The effects of two doses of haloperidol on the dopamine synthesis in rat corpus striatum were shown in Fig. 3, Fig. 4. After haloperidol administration (3.3×10^{-4} M), the dopamine synthesis rate was reduced to approximately 78% of the control value (Fig. 3). Doses of 2.76×10^{-4} M, 1.06×10^{-4} M, 6.6×10^{-5} M of halorperidol also reduced synthesis rate to about 70%, 87%, 90% of the control values respectively.

The inhibitory effects of dopamine synthesis occurred about 10–30 mintues after haloperidol administration. The maximum effect of depression of dopamine synthesis occurred about 80–90 minutes after haloperidol administration. Haloperidol administrated with lower doses of 3.3×10^{-5} M, 1.99×10^{-5} M and 6.6×10^{-6} M reversed the effects to increase the dopamine synthesis rate to about 107%, 116% and 110% (Fig. 4) respectively. The excitatory effects of dopamine synthesis occurred about 50 minutes after haloperidol administration. Haloperidol administration with dose of 3.3×10^{-6} M produced no significant effect on dopamine synthesis. The effects of haloperidol on dopamine synthesis as a function of haloperidol concentration were summarized in Fig. 5.

DISCUSSION

Morphine and haloperidol had been shown to increase dopamine turnover in the corpus striatum. However, those data were obtained

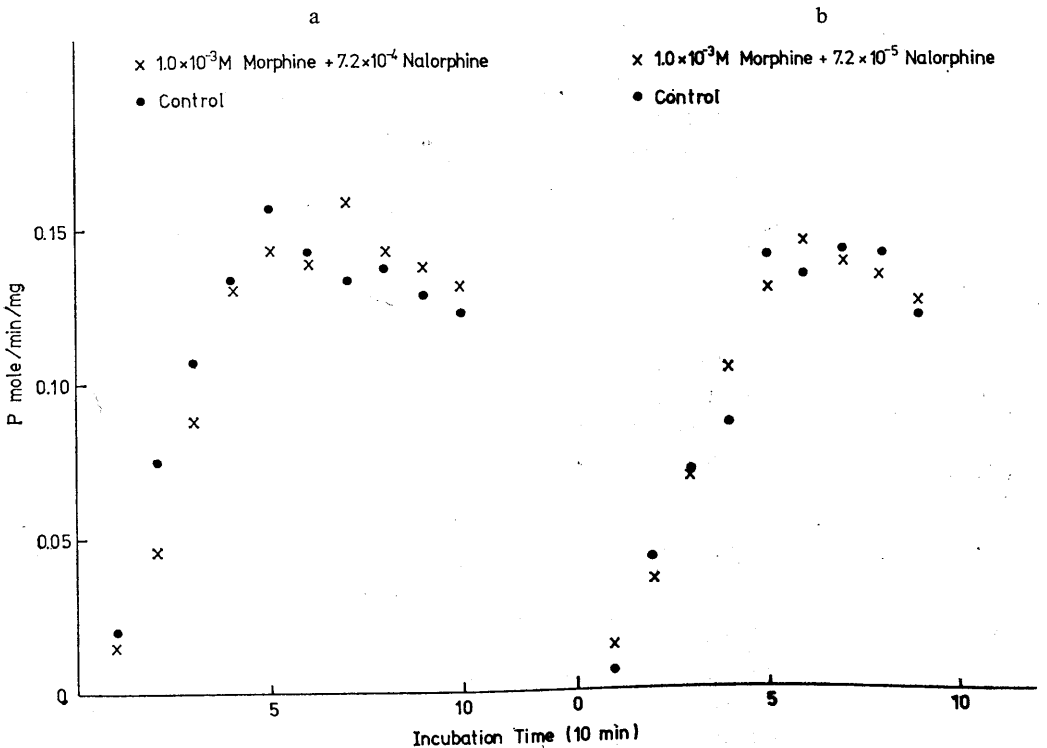

Fig. 2. Nalophine blocked the stimulating effect of morphine on the dopamine biosynthesis.

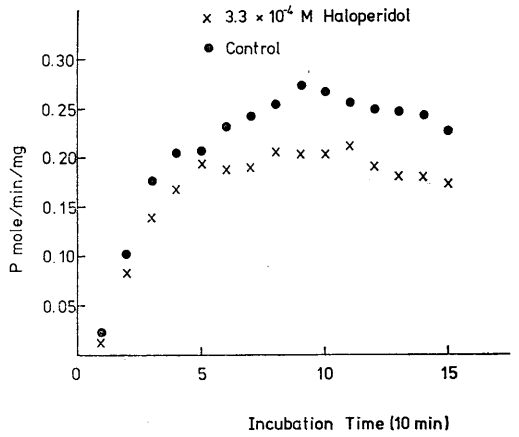

Fig. 3. Haloperidol at high concentration inhibited dopamine biosynthesis.

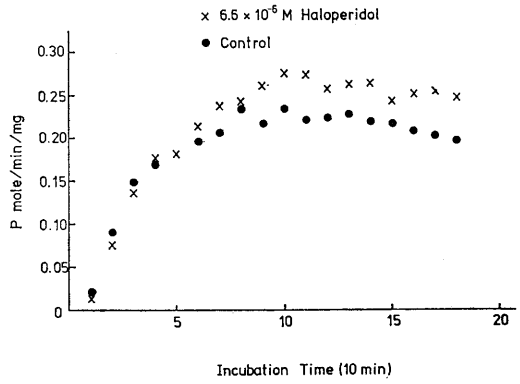

Fig. 4. Haloperidol at low concentration stimulated dopamine biosynthesis.

in the *in vivo* experiments and no clear site of the action could be identified. The present study showed that both morphine and haloperidol significantly changed dopamine synthesis rate in the ruptured nerve-ending preparation isolated from rat corpus striatum.

The principle mode of action of morphine in producing increase effect of dopamine synthesis was recently suggested to be in the dopamine presynaptic receptor by way of GABA mediation[8]. According to Sharman[23], Kuschinsky *et al.*[16] and Papeschi *et al.*[21], morphine may lead to a diversion of newly synthesized dopamine from storage sites to sites of catabolism. However, there is no suggestion for any specific

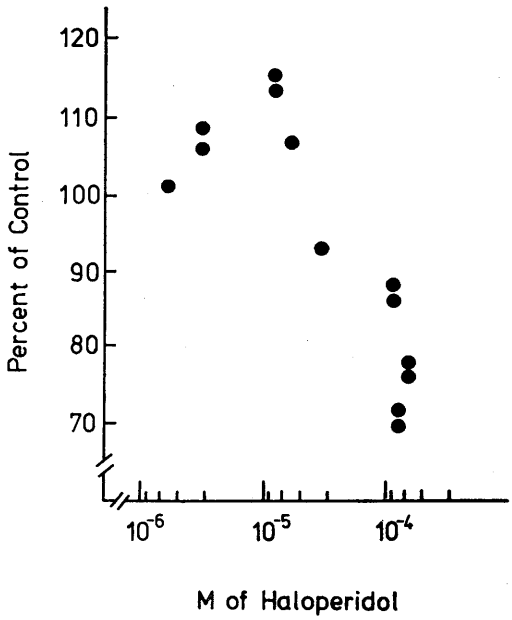

Fig. 5. Effect of haloperidol on the rate of dopamine biosynthes as a function of haloperidol concentration.

mechanism that may be responsible for such a diversion. We therefore suggest that morphine function may be the activation of the reactions catalyzed by COMT (Catechol-O-Methyltransferase) and MAO (Monoamine Oxidase) after the binding of presynaptic receptors. The results of such a stimulation at presynaptic nerve ending of dopamine will be (1) increase dopamine turnover rate[21] and reduce the transmitted dopamine, (2) increase the dopamine synthetic rate[7, 10, 24] due to remove of the feedback inhibition on the tyrosine hydroxylase, (3) increase the amount of the HVA and DOPAC[9, 18, 17]. This sugestion was further supported by Biggio *et al.*[2], who found that enkephalin had similar effects on dopamine metabolism. More investigation about the similarity between enkephalin and morphine will be needed to clarify the properties of these pre-synaptic receptors.

Although scopolamine[22] or atropine[17] failed to prevent catalepsy produced by morphine, our own observation[26] showed that acetylcholine significantly stimulate dopamine synthesis. This mutual regulation of acetylcholine and

dopamine may be related to the tolerance or long term effect of morphine[8].

Hornykiewics[13] reported that dopaminergic antagonist, neuroleptics, might produce its effect by the blockade of the dopamine-sensitive sites on the striatal neuron. Dopamine-sensitive adenylate cyclase was not inhibited *in vitro* by opiates while haloperidol blocked the stimulation by dopamine of adenylate cyclase activity in the caudate nucleus of the striatum[5,15]. It has long been suggested that presynaptic dopamine or autoreceptor may have an important physiological role in the modulation of dopamine synthesis and release. Following long term treatment of rats with a phenothiazine or haloperidol, an increase in the presynaptic sensitivity has also been observed[11]. However, our finding that haloperidol changed the dopamine biosynthesis in synaptosomal preparation and this effect can not be prevented by nalorphine suggested that haloperidol may have direct effect on the presynaptic dopamine neuron and the effects were not from the pre-synaptic opiate receptor.

A dose dependence of both excitatory and inhibitory effect on the dopamine biosynthesis by haloperidol was a new discovery. Both stimulation and inhibition we found can not due to neuronal or hormonal feedback mechanism. The possible mechanism of direct stimulation on presynaptic dopamine receptors are under study.

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Morphine 和 Haloperidol 對神經扣結前 Dopamine 合成之影響

徐百川 蔣 宜 王唯工

本篇報告 Morphine 和 Haloperidol 對神經扣結前之 Dopamine 生理合成的影響，Dopamine 之合成速率以連續測量神經扣結中 L-(1-^{14}C) 酪氨酸代謝所產生之 $^{14}CO_2$ 來表示。Morpine 有刺激 Dopamine 合成之效應，此一刺激之效應可被 Nalorphine 所對抗，所以認爲 Morphine 之作用是在神經扣結前之 Opiate receptor，其與 Opiate receptor 結合後之作用推斷爲加速 COMT 與 MAO 之催化作用，Haloperidol 在低劑量時刺激 Dopamine 之合成，在高劑量時抑制 Dopamine 之合成。