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On the mechanism of morphine action on rat striatal dopamine metabolism

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Morphine is known to decrease motor activity and to increase striatal dopamine metabolism in rats [1]. Several hypotheses have been invoked to explain these effects which are presumably mediated by activation of opiate receptors in the CNS since they are abolished by the morphine antagonist naloxone. Kuschinsky [1] and Celsen and Kuschinsky [2] have proposed that morphine causes a diversion of intraneuronal dopamine to nonfunctional metabolism and decreases the rate of dopamine release, actions consistent with both the akinesia and increased metabolism of dopamine. Others [3, 4] have proposed that the morphine-induced increase of dopamine metabolism may be due to indirect stimulation by morphine of dopaminergic impulse flow. Thus, turning behavior, induced by either an agonist of dopamine receptors, apomorphine, or an antagonist, haloperidol, in rats with unilateral striatal lesions, is inhibited by morphine [3]. Also, the morphine-induced increase of dopaminergic impulse flow has a slow onset of action compared with known direct acting dopamine agonists or antagonists [4]. Costa *et al.* [5] have proposed that a pre-synaptic enkephalinergic action on dopamine nerve terminals enhances release of dopamine per impulse. According to their proposal, apomorphine or haloperidol may either inhibit or activate a cholinergic to enkephalinergic loop feeding back on the dopamine nerve ending to either inhibit or activate tyrosine hydroxylase. The first two hypotheses are compatible in that decreased neurogenic release of dopamine would be expected to reflexly increase impulse flow in the nigral-striatal pathway. All three proposals are in harmony with the observed additive effects of morphine and chlorpromazine, a dopamine receptor blocker, to increase striatal dopamine metabolism [6].

The first two hypotheses mentioned above can be tested by using an impulse-dependent releaser of dopamine. Such a drug is the potent CNS stimulant amfonelic acid (AFA) [7]. The release of dopamine per impulse is enhanced by AFA such that, if haloperidol is co-administered in order to maintain impulse flow in the presence of AFA, a potentiated increase of dopamine metabolite concentrations results [8]. Inhibitors of dopamine impulse flow (i.e. apomorphine or γ-butyrolactone) prevent the synergism of these two drugs [8]. Shore *et al.* [7] proposed that AFA and other nonamphetamine stimulants enhance the transfer of dopamine from a large storage pool to a releasable pool so that greater amounts of transmitter are released per impulse. If morphine decreases the neurogenic release of dopamine and diverts dopamine to non-functional metabolism, then AFA, which alone causes a slight increase of dopamine metabolism [8], would be expected to either inhibit the morphine-induced increase of dopamine metabolism or show an additive effect, but not a marked synergism. However, if morphine is indirectly driving impulse flow [4], AFA would be expected to exhibit a supra-additive effect on dopamine metabolism when combined with morphine in a manner analogous to the interaction of AFA with haloperidol.

The hypothesis of Costa *et al.* [5] that the presumed dopamine autoreceptor is actually a pre-synaptic opiate receptor, activated by a cholinergic–enkephalinergic loop, can be tested by using the γ-butyrolactone (GBL) model for studying dopamine pre-synaptic receptors. Administration of GBL inhibits nigral dopamine neuronal impulse flow and increases dopamine levels and tyrosine hydroxylase activity in the striatum [9]. This effect of GBL may be due to either decreased extraneuronal dopamine available for binding to pre-synaptic autoreceptors [9] or activation of pre-synaptic enkephalinergic neurons subsequent to decreased dopaminergic activity [5]. The latter possibility can be tested by observation of the effects of pretreating with either morphine, which should enhance, or naloxone, which should inhibit the effect of GBL on dopamine levels.

Female Sprague–Dawley rats (Holtzman, Madison, WI), 200–250 g, were given various drugs s.c., except for GBL which was given i.p., and killed by chloroform asphyxiation at the times noted in the tables. The brains were rapidly removed, chilled in ice-cold saline, and the corpora striata dissected out and frozen over dry ice. Assays were performed the same day. Dihydroxyphenylacetic acid (DOPAC) was measured by organic extraction and fluo-

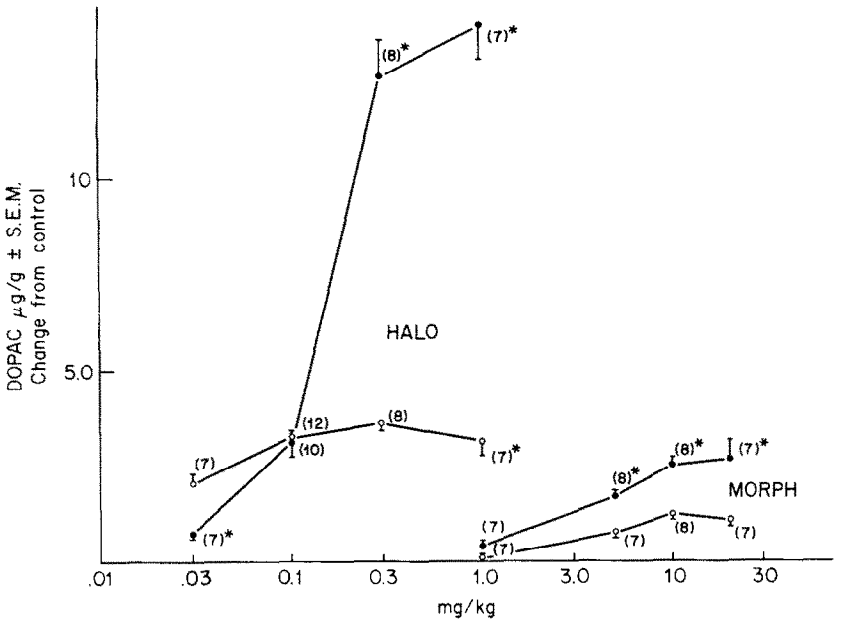

Fig. 1. Effects of various doses of haloperidol or morphine with (●) or without (○) 2.5 mg/kg AFA. The concentrations of DOPAC shown are changes in μg/g ± S.E.M. from saline control (1.43 ± 0.08 μg/g). The asterisk (*) denotes significant differences from test drug alone where P is at least less than 0.005 (Student's *t*-test). Numbers in parentheses represent the number of animals in each group.

rophor development [10]. Dopamine was measured fluorometrically according to the method of Neff and Costa [11].

Drugs used were: amfonelic acid (Sterling–Winthrop Research Institute, Rensselaer, NY), γ-butyrolactone (Sigma Chemical Co., St. Louis, MO), haloperidol (McNeil Laboratories, Ft. Washington, PA), morphine sulfate (Merck & Co., Rahway, NJ), and naloxone–HCl (Endo Laboratories, Garden City, NY). All doses refer to the free acid or base.

Figure 1 shows the dose–response curves for morphine and haloperidol, alone or in combination with 2.5 mg/kg AFA, with change in μg/g DOPAC as the response. Maximal elevation of DOPAC levels was obtained at 0.3 mg/kg of haloperidol or 10 mg/kg of morphine, but haloperidol elicited a much larger increase in DOPAC concentration than did morphine (252 vs 83 per cent). An additive effect of AFA combined with morphine on DOPAC levels was observed with those doses of morphine that increased dopamine metabolism alone (5–20 mg/kg, but not 1 mg/kg). A low dose of haloperidol (0.03 mg/kg) did not reduce the hyperactivity seen after AFA, and the rise in DOPAC concentrations seen after the neuroleptic was inhibited by AFA. The expected synergism with dopamine metabolism [8] was seen only after doses of haloperidol (0.3 or 1.0 mg/kg) which completely inhibited the behavioral stimulant effects of AFA. The maximal response obtained with haloperidol in combination with AFA was much greater than with morphine in combination with AFA (Fig. 1). Hyperactivity and stereotypy after AFA were observed when AFA was combined with 1.0 or 5.0 mg/kg of morphine. With either of the two larger doses of morphine, a slow sustained gnawing behaviour was elicited by AFA. Morphine alone caused sedation, except for the 1.0 mg/kg dose which was without effect either behaviorally or biochemically.

To determine whether the effects of morphine on dopamine metabolism are mediated by opiate receptors, rats were pretreated with 2.0 mg/kg of naloxone, a potent narcotic antagonist. Naloxone alone did not alter DOPAC concentrations, but did prevent the morphine-induced increase of DOPAC levels (Table 1). Similarly, naloxone prevented the additive effect of AFA and morphine ($P > 0.1$, compared to AFA alone). Neither naloxone nor morphine influenced the synergism of AFA and haloperidol, as shown in Table 2.

Additional experiments were performed to determine whether the presumed pre-synaptic autoreceptor is mediated by a pre-synaptic enkephalin neuron. As shown in Table 3, GBL (750 mg/kg) produced the expected [9] increase of dopamine concentration in rat striatum. Apomorphine, a dopamine agonist, inhibited this response to GBL, but 30-min pretreatments with either morphine or naloxone were without any influence on the 30-min accumulation of striatal dopamine caused by GBL. In harmony with these results, Andén and Grabowska-Andén [3] reported that morphine altered neither the GBL-induced activation of *in vivo* tyrosine hydroxylase activity nor the inhibition of the GBL effect by apomorphine.

Although morphine is known to increase striatal dopamine metabolism (see Ref. 1), the mechanism(s) involved is not well elucidated. Enkephalins are present in the striatum [12], and one-third of opiate receptors in the striatum may be located on dopaminergic nerve terminals, as evidenced by loss of leu^5-enkephalin binding after lesions of the substantia nigra [13]. Thus, a local action of morphine on striatal dopaminergic terminals seems likely and has been suggested as the mechanism of morphine-induced elevation of dopamine metabolism [5, 13]. Significantly, intraventricular administration of (D-ala^2)-met-enkaphalin [14] or intra-caudate injections of morphine or β-endorphin [15] increase striatal dopamine metabolism, which further emphasizes a local effect of morphine in the neostriatum.

However, pre-synaptic opiate receptors on dopaminergic axons are not necessarily the site of action of morphine. Opiate peptides have a wide distribution through the CNS [16]. In the basal ganglia, a striopallidal leu-enkephalin pathway has been demonstrated [17]. Intra-caudate injec-

Table 1. Effects of morphine, naloxone, AFA or their combination on rat striatal dopamine metabolism*

Treatment	DOPAC (μg/g ± S.E.M.)
Control	1.43 ± 0.09 (12)
Morphine	2.61 ± 0.06† (8)
Naloxone + morphine	1.59 ± 0.08 (7)
AFA	1.95 ± 0.05‡ (8)
Morphine + AFA	3.93 ± 0.21† (8)
Naloxone + morphine + AFA	2.22 ± 0.19† (8)
Naloxone	1.46 ± 0.06 (8)

* Morphine (10 mg/kg, s.c.) or AFA (2.5 mg/kg, s.c.) was injected 90 min before death and naloxone (2.0 mg/kg, s.c.) 95 min before death. Numbers in parentheses represent the number of animals in each group.

† Different from control, $P < 0.01$ (Dunnett's t-test).

‡ Different from control, $P < 0.05$ (Dunnett's t-test).

Table 3. Effect of γ-butyrolactone, alone or in combination with narcotic drugs, on rat striatal dopamine concentrations*

Treatment	Dopamine (μg/g ± S.E.M.)
GBL	20.02 ± 0.68 (12)
Apomorphine + GBL	16.62 ± 0.38† (8)
Morphine + GBL	20.86 ± 0.67 (8)
Naloxone + GBL	20.08 ± 0.49 (6)
Controls	13.00 ± 0.88† (12)

* Apomorphine (1.0 mg/kg), morphine (10 mg/kg) or naloxone (2.0 mg/kg) was injected s.c. 30 min before GBL (750 mg/kg, i.p.) and the animals were killed 30 min later. Numbers in parentheses represent the number of animals in each group.

† Different from GBL alone, $P < 0.01$ (Dunnett's t-test).

tions of morphine or β-endorphin alter γ-aminobutyric acid (GABA) turnover in the caudate and the globus pallidus [15]. These effects do not appear to be mediated by the nigral-striatal pathway, but rather reflect a direct effect on GABA neurons [15]. In addition to enkephalinergic systems in the neostriatum, there are opiate receptors in the substantia nigra which may be located on pre-synaptic terminals originating rostrally, as evidenced by loss of these receptors after hemisection [18]. Intra-nigral injection of morphine does not alter striatal tyrosine hydroxylase activity, which suggests the nigra is not the site of activation by systemic morphine, but inhibits systemic haloperidol-induced activation of striatal tyrosine hydroxylase [18]. Thus, opiate effects on GABA turnover, modulation of synaptic inputs to the substantia nigra, or other systems could mediate the actions of systemic morphine on striatal dopamine metabolism.

The potent CNS stimulant AFA was used to differentiate possible mechanisms of morphine-induced increased dopamine metabolism. That AFA is dependent upon impulse flow to enhance dopamine release and metabolism [7, 8] is further supported by the haloperidol dose–response curve of Fig. 1. The combination of AFA with a dose of haloperidol insufficient to inhibit the stimulant effects of AFA results in DOPAC concentrations lower than with haloperidol alone, but greater than control. The large release of dopamine by AFA, which alone inhibits dopaminergic impulse flow [19], apparently overcomes the blockade of dopamine receptors by small doses of haloperidol. When sufficient haloperidol is used to inhibit AFA-induced behavioral stimulation and reduction of impulse flow [19], a marked synergism between these two drugs on dopamine metabolism is observed (Fig. 1, [8, 19]). However, AFA exhibits only an additive effect on dopamine metabolism when combined with morphine. There occurs neither a reduction at low doses nor a marked synergism at high doses of morphine. Even when hyperactivity and stereotypy occurs, as is the case with 5.0 mg/kg morphine and 2.5 mg/kg AFA, a reduction of morphine induced DOPAC elevation is not observed (Fig. 1). If morphine is driving impulse flow, the mechanism would seem independent of the striatal-nigral feedback loop, as suggested by others [3, 4]. Thus, morphine may not be able to overcome inhibition of nigral impulse flow by AFA and synergism on dopamine metabolism does not occur.

Table 2. Effects of morphine or naloxone on the increased dopamine metabolism induced by haloperidol and AFA*

Treatment	DOPAC (μg/g ± S.E.M.)
Haloperidol + AFA	14.12 ± 0.89 (8)
Morphine + haloperidol + AFA	15.24 ± 0.66 (8)
Naloxone + haloperidol + AFA	13.10 ± 0.73 (8)
Haloperidol	5.03 ± 0.20† (8)

* Haloperidol (0.3 mg/kg) and AFA (2.5 mg/kg) were injected s.c. 90 min before death and morphine (10 mg/kg) or naloxone (2.0 mg/kg) 95 min before death. Numbers in parentheses represent the number of animals in each group.

† Different from haloperidol + AFA, $P < 0.01$ (Dunnett's t-test).

A pre-synaptic effect of morphine to increase dopamine release seems an unlikely explanation of the increased dopamine metabolism. If morphine enhances dopamine release by a pre-synaptic mechanism as suggested by Costa *et al.* [5], a decreased rate of nigral impulse flow would be expected, which is the opposite result of actual single cell recordings [4]. The data of Table 3 (also Ref. 3) further suggest that the presumed pre-synaptic dopamine autoreceptor is not mediated by opiate receptors as neither morphine nor naloxone has any effect on the responses to GBL. Furthermore, morphine actually inhibits *in vitro* release of dopamine from striatal slices [2].

On the other hand, if morphine decreases the rate of dopamine release yet increases dopamine metabolism [1, 13], then combining AFA with morphine could be expected to have one of two effects on dopamine metabolism. Either the two drugs should antagonize each other or, if morphine and AFA are acting through separate mechanisms, an additive effect should be observed. This latter result is the observation reported in Fig. 1 and Table 1. The lack of antagonism of morphine by AFA suggests that the increased dopamine metabolism caused by morphine is not mediated by the sequence of decreased release leading to loss of dopamine at the pre-synaptic autoreceptor causing enhanced tyrosine hydroxylase activity, as proposed by Schwartz and co-workers [13, 20]. Inhibition of dopamine reuptake by AFA [7] should reverse this process by increasing the amount of dopamine in the synaptic cleft. The additive effect of these two drugs suggests independent mechanisms for enhancing dopamine metabolism. These results are consistent with the hypothesis that morphine diverts dopamine to nonfunctional metabolism [1, 2]. Whether morphine-induced enhanced dopaminergic impulse flow [4] is due to decreased dopamine release and subsequent feedback activation of the nigral-striatal path-

way or to effects of morphine at other sites within the CNS is unclear. It is possible that the two effects of morphine, enhanced dopamine metabolism and impulse flow, reflect activation of opiate receptors at two unrelated sites, and the amount of dopamine released will reflect the sum of these two phenomena. To separate these sites of action will be difficult because of the multiplicity of opiate agonist effects in the extrapyramidal system [13, 15, 16, 18].

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Effect of ethanol administration on free proline and glutamate in the intact rat liver

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The concentration of free proline is considered to be significant in the formation of collagen [1, 2]. Earlier we observed that the synthesis of proline was increased during the incubation of liver homogenates obtained from ethanol-treated rats or of liver slices when ethanol was added *in vitro* [3]. The conclusions on the role of glutamate as a precursor of free proline in liver are variable [1, 3, 4].

In humans, Kershenobich *et al.* [5] demonstrated a correlation between the concentrations of free proline and collagen in liver. Siegel *et al.* [6] studied alcoholics and did not find any change from normal in serum proline but, following ethanol load, an elevation of proline was found. Similarly, Shaw and Lieber [7] observed in baboons that after a long-term administration of ethanol, proline was depressed in post-prandial serum. Mezey *et al.* [8] observed in rat liver an increase of free proline after ethanol feeding. In our own study [9] we also found that a long-term administration of ethanol to the rats increased the concentration of free proline in liver, although by 10–12 per cent only, on average. Mørland *et al.* [10] did not observe any change in hepatic free proline in rats after long-term ethanol feeding, while Forsander (personal communication) found a decrease in liver proline after a 2-month treatment with ethanol, although there was an increase in liver glutamate.

The purpose of this work was to find out whether a single-dose administration of ethanol will cause an increase of free proline in liver *in vivo*, and to discover what are the simultaneous changes in liver glutamate. There is a shift in the redox balance towards the reducing side during the catabolism of ethanol in liver [11]. This change favours the synthesis of proline. Therefore, we wanted to see whether by diminishing this change in the redox balance caused by the oxidation of ethanol we could prevent the increase in the amount of proline. For that purpose the rats were treated with 4-methylpyrazole, a potent inhibitor of alcohol dehydrogenase [12]. Attempts to influence the redox balance were made also by giving methylene blue to the rats.

Experimental

Treatment of animals. Male Sprague–Dawley rats, aged 2–4 months, and fasted for about 40 hr, were given ethanol by stomach tube, usually 2 g per kg body wt as a 25% (v/v) aqueous solution. In the preliminary experiments, ethanol doses of 4 and 8 g per kg were also used. The control rats received corresponding volumes of water.

4-Methylpyrazole was injected i.p. 10 min before the administration of ethanol. On the basis of the preliminary experiments with doses from 10 μmoles to 2.44 mmoles per kg body wt, the dose adopted for use was 0.2 mmoles or 16.4 mg per kg.

Methylene blue was given 5 or 10 mg per kg as an i.p.