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THE EFFECTS OF MORPHINE ON THE ACCUMULATION OF HOMOVANILLIC AND 5-HYDROXYINDOLEACETIC ACIDS IN THE CHOROID PLEXUS OF RATS

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1 Choroid plexus obtained from the lateral ventricles of the rat actively accumulated homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA).

2 Morphine 5×10^{-6}M to 5×10^{-4}M potentiated 5-HIAA accumulation but did not affect HVA accumulation. Levorphanol and dextrorphan had little effect.

3 Naloxone at high concentrations inhibited both HVA and 5-HIAA accumulation.

4 Glutamic acid, glycine, and arginine also decreased 5-HIAA accumulation, but lysine, tryptophan, and aspartic acid had no effect.

5 Probenecid, naloxone, arginine, glycine, and tryptophan blocked the increase of 5-HIAA accumulation induced by morphine.

6 Acute or chronic morphine treatment did not increase the accumulation of 5-HIAA.

7 These results suggest that the increase of 5-HIAA or HVA in brain by morphine is not due to the inhibition of the elimination of these metabolites from the choroid plexus.

Introduction

Several neurotransmitters have been implicated in the pharmacological actions of morphine, and numerous attempts have been made to elucidate the mechanism of action of morphine involving changes in levels of biogenic amines such as 5-hydroxytryptamine (5-HT) or catecholamines (see reviews of Way & Shen, 1971; Takemori, 1974, and many others). Since the turnover of biogenic amines in neurones may reflect neuronal activity, the effects of morphine on their turnover have been studied by a variety of methods.

However, the results of these investigations are often equivocal because different methods were used. One method of studying the turnover of 5-HT is to measure 5-hydroxyindoleacetic acid or homovanillic acid accumulation after inhibiting their transport by probenecid (Neff, Tozer & Brodie, 1967). This method has its advantages; it is simple and can be applied to human beings. However, if a drug *per se* has an effect on the transport of acidic metabolites in the brain, the use of this method to study 5-HT or dopamine turnover may produce misleading results. Since the choroid plexus is considered to be one of the active sites of their transport, and accumulation of acidic metabolites in the choroid plexus *in vitro* could reflect such activity, we examined the effects of morphine in this system. This choice was supported by the fact that, after the injection of morphine, histological examination of the choroid plexus revealed the appearance of numerous vacuoles, suggesting intense secretory activity and pronounced ischaemia (Wajda, Wajda, Manigault & Steiner, 1974).

Methods

5-Hydroxyindoleacetic acid (5-HIAA), labelled with ^{14}C in the carboxyl position (specific activity 22.1 mCi/mmol) was obtained from New England Nuclear Corp., Boston, Mass. [G-^{3}H]-homovanillic acid (specific activity 585 mCi/mmol) was obtained from Commissariat a l'Energie Atomique, Gif-sur-Yvette, France. The organic solvents which dissolved these radioactive compounds were removed by vacuum evaporation, and subsequently the compounds were dissolved in 0.01 N HCl or distilled water. The incubation medium was modified Krebs-Ringer phosphate solution of the following composition (mM): NaCl 135, KCl 5, $MgSO_4$ 1.3, $CaCl_2$ 0.5, glucose 12, and NaH_2PO_4-Na_2HPO_4 buffer 10. This incubation medium has been used in previous studies dealing with accumulation in the choroid plexus of morphine (Takemori & Stenwick, 1966) and of methadone (Huang & Takemori, 1975). The incubation medium was bubbled with O_2 for 1 h

before use; the pH of the medium was 7.4. 5-HIAA or homovanillic acid (HVA) and other chemicals were added to 3 ml of incubation medium just before incubation.

Female Sprague-Dawley rats (200 to 250 g) were used in all experiments. The schedule of chronic morphine treatment was adapted from Cox, Ginsburg, Willis & Davies (1975). Rats were treated with morphine sulphate twice daily for up to 7 days. Rats were decapitated 1 to 6 h after the last dose of morphine (200 mg/kg), and their brains rapidly exposed. Before incubation, the choroid plexus was isolated and placed in a cold moist glass chamber with filter paper soaked with 0.9% w/v NaCl solution (saline). Not more than 5 min elapsed from the isolation of the choroid plexus to the incubation. The incubation was performed at 37°C; the water bath was shaken at 60 rev/minute. Air was replaced by O_2, and after various time intervals the choroid plexus was removed from the medium and washed twice with ice-cold non-radioactive medium; the excess of medium in the choroid plexus was removed by drawing it across a glass slide as described by Cserr & Van Dyke, 1971. After weighing, the choroid plexus was transferred to the counting vials and the tissue dissolved in 0.5 ml of Soluene (Packard Inst. Co., Downer Grove, Illinois, U.S.A.). Their radioactivity and the radioactivity of the incubation media were measured by liquid scintillation spectrometry. Scintillation fluid consisted of PPO (5 g) and dimethyl POPOP (0.25 g) in 1 l of toluene. The counting efficiency was about 85% for ^{14}C and 30% for ^{3}H.

The calculation of 5-HIAA accumulation (tissue to medium ratio) was d/min (disintegrations per minute) per wet choroid plexus weight (g) divided by d/min of incubation medium (ml) and multiplied by the factor of 0.8 (Quay, 1966). Neither metabolism nor the binding of 5-HIAA in the choroid plexus was considered (Sampath & Neff, 1974). HVA was not metabolized significantly by the choroid plexus during 60 min incubation. The data were statistically analyzed by Student's t test (paired and two tail) because control and experimental choroid plexus were from the same rats. Significant value is P less than 0.05.

Results

Accumulation of 5-hydroxyindoleacetic and homovanillic acids by the choroid plexus

Choroid plexus from rat lateral ventricle accumulates 5-HIAA and HVA against an apparent concentration gradient, as indicated by the fact that tissue to medium ratio was always greater than unity during the incubation period (5–60 minutes). The accumulation of 5-HIAA increased continuously with the incubation time while HVA accumulation was maximal at about 30 min then gradually decreased so that tissue to medium ratio after 60 min was similar to that at 5 minutes.

In the subsequent experiments the incubation times for 5-HIAA and HVA were 20 and 15 min

Table 1 Effect of opiates on the accumulation of 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) in choroid plexus of rat

Compound	*Final concentration (M)*	*Mean % of control* 5-HIAA	HVA
Morphine	5×10^{-3}	—	105.7 ± 6.5
	5×10^{-4}	122.2 ± 6.8*	101.1 ± 8.0
	5×10^{-5}	136.1 ± 5.5*	100.8 ± 6.3
	5×10^{-6}	119.4 ± 4.2*	—
Levorphanol	5×10^{-4}	100.3 ± 6.0	86.6 ± 6.8
	5×10^{-5}	113.3 ± 2.0*	88.6 ± 12.4
	5×10^{-6}	104.8 ± 6.6	—
Dextrorphan	5×10^{-4}	97.6 ± 5.1	96.3 ± 7.0
	5×10^{-5}	107.3 ± 4.6	93.1 ± 5.9
Naloxone	5×10^{-3}	—	62.6 ± 7.2*
	5×10^{-4}	83.4 ± 4.6*	82.5 ± 4.5*
	5×10^{-5}	89.4 ± 8.6	101.7 ± 10.4
	5×10^{-6}	97.4 ± 3.1	—

The accumulation was measured by tissue to medium ratio of choroid plexus incubated in 3.6×10^{-7} M of 5-HIAA for 20 min and 2.7×10^{-7} M of homovanillic acid for 15 min at 37°C under O_2. Each value represents a mean with s.e. mean of five paired experiments.

*$P < 0.05$.

respectively since they coincided with the middle of the linear increase of accumulation.

Effect of opiates on the accumulation of 5-hydroxyindoleacetic and homovanillic acids in vitro

In the presence of morphine in the incubation medium (5×10^{-6} to 5×10^{-4}M) the accumulation of 5-HIAA in the choroid plexus increased significantly (about 30%). Levorphanol was less effective and dextrorphan was without any effect. In the presence of naloxone (5×10^{-4}M) the accumulation of 5-HIAA was decreased. The effect of opiates on the uptake of HVA by choroid plexus was different from the effect of 5-HIAA uptake. Morphine, levorphanol, and dextrorphan were without any effect. Naloxone at high concentrations inhibited the accumulation of HVA (Table 1).

Pretreatment with morphine and the accumulation of 5-hydroxyindoleacetic acid

Choroid plexus obtained from rats injected with a single dose of morphine (10 or 40 mg/kg) and killed 1 h after the injection showed no difference from that of saline-treated rats; in the two groups the tissue to medium ratio after 3 or 20 min incubation was the same. After morphine injections, the incubation with probenecid resulted in a decreased uptake (Table 2). After chronic morphine treatment for one week and

Table 2 Accumulation of 5-hydroxyindoleacetic acid (5-HIAA) in the choroid plexus isolated from morphine-treated rats

Experimental conditions	*Incubation time (min)*	*T/M ± s.e.**
Control (saline-treated)	3	1.6 ± 0.1
	20	3.7 ± 0.2
Morphine 10 mg/kg for 1 h	3	1.6 ± 0.1
	20	3.8 ± 0.2
Morphine 40 mg/kg for 1 h	20	3.9 ± 0.1
Morphine 40 mg/kg for 1 h and incubation with 1×10^{-4}M of probenecid	20	2.6 ± 0.1**
Morphine 20 mg/kg increased to 200 mg/kg twice daily for 7 days; 1 h after last dose	20	3.9 ± 0.4
Morphine 20 mg/kg increased to 200 mg/kg twice daily for 7 days; 6 h after last dose	20	4.0 ± 0.2

*Tissue to medium (T/M) ratio was based on the uptake of 3.6×10^{-7}M of 5-HIAA for 20 min at 37°C under O_2. Each value represents a mean with s.e. mean of four experiments.

**$P < 0.05$.

Table 3 Effect of amino acids and probenecid on the accumulation of 5-hydroxyindoleacetic acid (5-HIAA) in the choroid plexus

Amino acid	*Tissue to medium ratios*		
	Control	*With amino acid**	*Mean % of control*
Arginine	3.8 ± 0.2	2.9 ± 0.1	76.9**
Lysine	3.8 ± 0.4	3.6 ± 0.1	94.7
Glycine	4.0 ± 0.3	3.1 ± 0.2	77.5**
Tryptophan	3.9 ± 0.1	4.0 ± 0.3	102.2
Aspartic acid	3.6 ± 0.3	3.3 ± 0.1	90.7
Glutamic acid	4.0 ± 0.4	3.4 ± 0.2	85.0**
Probenecid	3.9 ± 0.1	2.6 ± 0.1	66.7**

*The final concentration of amino acids was 1×10^{-4}M. Each value represents mean with s.e. mean of five experiments.

**$P < 0.05$.

also upon withdrawal from morphine, the choroid plexus showed the same accumulation ratio as the controls.

Effects of amino acids

Six amino acids were tested for their effects on the accumulation of 5-HIAA by the choroid plexus. Arginine, glycine, and glutamic acid were inhibitory at a concentration of 1×10^{-4}M (about 20%), whereas lysine, tryptophan, and aspartic acid were without effect (Table 3). The results suggest that the inhibitory effect of amino acids on the accumulation of 5-HIAA does not depend on the pH of the amino acid. The effect of amino acids was not present at low concentrations; for instance, glycine at a concentration of 1×10^{-6}M was without effect.

Interaction of morphine and other compounds on 5-hydroxyindoleacetic acid accumulation

Naloxone, aspartic acid, and tryptophan were tested together with morphine at concentrations that were without effect in the absence of morphine. However, they were able to block the increase in the accumulation of 5-HIAA induced by morphine alone (Table 4). Probenecid and glycine which decreased the accumulation of 5-HIAA (Table 3) inhibited the usual increase in accumulation induced by morphine.

Discussion

The present results indicate that the choroid plexus isolated from the rat lateral ventricles can actively accumulate 5-HIAA and HVA and these uptake mechanisms differ in their response to morphine which potentiated the accumulation of 5-HIAA but had no effect on the accumulation of HVA. However, the reasons for the differences and for the appearance of the vacuoles in the ependymal cells of the choroid plexus after morphine were not elucidated by this investigation.

Since levorphan, a more potent analgesic than morphine, was less potent in stimulating the accumulation of 5-HIAA, it seems that the potentiation of 5-HIAA accumulation has no relation to the analgesic effects of opiates. Nevertheless, naloxone, which antagonizes the analgesic action of opiates, was able to block this potentiation of 5-HIAA accumulation produced by morphine.

Goodlet & Sugrue (1974) reported that morphine was able to produce an increase in the concentration of 5-HIAA in the brain, whereas other narcotics such as methadone, pentazocine, and pethidine or naloxone were inactive. They suggested that only morphine is involved in serotoninergic activity of the brain. More recent study of inhibitory effect of fluoxetine on 5-HT uptake further supports their conclusion (Sugrue & McIndewar, 1976). Our results also support this conclusion because among four morphine analogues studied, only morphine increased the accumulation of 5-HIAA in the choroid plexus.

We have confirmed that probenecid was very effective in inhibiting 5-HIAA uptake in the isolated choroid plexus; it also inhibited the accumulation of 5-HIAA in the choroid plexus obtained from morphine-treated rats. This result would be expected if the increase of 5-HIAA by morphine were due to the inhibition of its transport since acute and chronic morphine treatments potentiate the increase of 5-HIAA in rat brain by probenecid (Haubrich & Blake, 1973; Papeshi, Theiss & Herz, 1974; Goodlet & Sugrue, 1974).

The increase of 5-HIAA accumulation in isolated choroid plexus by morphine seems to be incompatible with the increase of 5-HIAA concentration in brain

Table 4 The interaction of morphine with other compounds in the accumulation of 5-hydroxyindoleacetic acid in the choroid plexus

	Tissue to medium ratios		
*Treatment with compound**	*Morphine alone*	*Morphine + compound*	*Mean % of morphine*
Probenecid	4.6 ± 0.2	2.6 ± 0.3	56.8**
Naloxone	4.5 ± 0.1	3.8 ± 0.2	86.1**
Aspartic acid	4.3 ± 0.4	3.5 ± 0.4	79.8**
Glycine	4.2 ± 0.2	3.7 ± 0.2	87.9**
Tryptophan	4.7 ± 0.4	3.6 ± 0.2	75.9**

Morphine was present in all incubations.

*The concentration of morphine and naloxone was 5×10^{-6}M; other compounds were 1×10^{-4}M. The conditions of incubation were as in Table 1. Each value represents mean with s.e. mean of five experiments.

**$P < 0.05$.

after acute or chronic morphine injection. Since the choroid plexus is one of the active sites for the elimination of 5-HIAA from the brain, morphine would be expected to act like probenecid and inhibit 5-HIAA accumulation in choroid plexus. It is possible that morphine inhibits other sites of the probenecid-sensitive transport system; it is known that the elimination of 5-HIAA by choroid plexus represents a minor process (Meek & Neff, 1973). The location of this probenecid-sensitive site is not likely to be in neurones, because Forn (1972) demonstrated that probenecid could not inhibit the efflux of 5-HIAA in brain slices. It is possible that the active site is located in brain capillaries.

Morphine-induced increase in 5-HIAA levels in the brain seems to be due to an increased rate of 5-HIAA formation rather than an inhibition of 5-HIAA efflux from brain (Haubrich & Blake, 1973; Goodlet & Sugrue, 1974). Our finding of an increase in the accumulation of 5-HIAA in the choroid plexus produced by morphine is in agreement with results of previous authors.

Since amino acids are present in the cerebrospinal fluid and certain amino acids, e.g. tryptophan (Ho, Brase, Loh & Way, 1975), aspartic acid (Koyuncuoglu, Gungor, Sagduyu & Eroglu, 1974), and glycine (Stern & Stern, 1974) prevent and modify morphine actions and physical dependence, the effects of these amino acids on 5-HIAA accumulation in choroid plexus were studied. At high concentrations, not far from the physiological concentration in cerebrospinal fluid, these amino acids decreased the accumulation of 5-HIAA and also blocked the increase of 5-HIAA accumulation produced by morphine. These results suggest that morphine cannot increase 5-HIAA uptake into the choroid plexus because of the presence of amino acids in the cerebrospinal fluid.

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