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Effects of Heroin and Naloxone on Cerebral Blood Flow in the Conscious Rat

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FULLER, S. A. AND E. A. STEIN. *Effects of heroin and naloxone on cerebral blood flow in the conscious rat.* PHARMACOL BIOCHEM BEHAV **40**(2) 339–344, 1991.—The widespread, heterogeneous distribution of opiate receptors and their endogenous ligands in the nervous system are reflective of the variety of central and systemic effects seen after opiate administration. Most neurons respond to either systemic or local opiate application with a decrease in firing rate, although increased neuronal activity has also been reported in such regions as the caudate, amygdala, ventral tegmentum, and substantia nigra. While regional metabolic studies have consistently reported neuronal suppression, some portion of this might be secondary to systemic hypercapnia. Using a brief blood flow marker, we recently reported a heterogenous increase in activity in more than half of the brain regions examined. To extend that study, we report herein the results of a dose-response and antagonist challenge experiment. Rats received an acute injection of one of the following: heroin (0.1, 0.3 or 1.0 mg/kg), naloxone (1.0 mg/kg), a cocktail of heroin (0.3 mg/kg) plus naloxone or saline. One min after drug administration, 160 μCi/kg [1-^{14}C] octanoate, a marker for cerebral blood flow, was delivered IV. Rats were sacrificed two min later, brains removed and prepared for autoradiography. Of the fifty-eight areas analyzed, heroin caused an increase in blood flow in the caudate, claustrocortex, laterodorsal thalamus and dentate gyrus. Decreases were found for the bed nucleus of the stria terminalis, preoptic area, basolateral nucleus of the amygdala, dorsomedial and paraventricular hypothalamus, entorhinal and cingulate cortices and dorsal raphe. Naloxone resulted in significant increases in the olfactory tubercle and paraventricular nucleus while decreases were seen in the cingulate and basolateral amygdala.

Heroin Naloxone Autoradiography Cerebral metabolic activity Rats

OPIATE agonist drugs have multiple actions both centrally as well as peripherally. Among their most prominent central effects include potent analgesia, sedation, alterations in body temperature and increases in food and water consummatory behavior (25). They also possess both direct reinforcing properties as well as the ability to enhance the affective quality of other reinforcing agents (e.g., brain stimulation reward) (35). It is likely that this multiplicity of actions requires activation at multiple independent brain sites or circuits and is mediated by several opioid receptor types (23,24). In addition, numerous nonopioid neurotransmitter systems including, but not limited to, a putative dopaminergic involvement in reinforcement (40) are engaged by these agents (34).

Many techniques have been employed to define central loci of opiate actions including single and multiunit electrophysiologic recording (8), direct brain microinjections of pharmacologic agents, selective neuroanatomic lesions or neurochemical measures (40). All of the above have greatly enhanced our understanding of the sites and mechanisms of opiate action. Their biggest drawback, however, is the limited number of brain areas that can be assayed from any one animal; in most cases only one or two regions. Autoradiographic techniques have the key property of being able to reflect activity in numerous and widespread regions simultaneously.

Local cerebral glucose utilization (lCGU) studies in experimental animals have consistently reported either decreases (1, 9, 18, 33) or no change (17,21) in neuronal activity after opiate administration from all brain regions analyzed. However, electrophysiologic recordings (5, 6, 15, 19, 26), as well as neurotransmitter turnover studies (34), have routinely observed regional increases and decreases in activity. In contrast to the lcgu studies, we recently reported heterogenous increases in regional cerebral blood flow (rCBF) 60 s after a single, acute dose of heroin in the conscious rat (37). Since opiate action is both dose and time dependent (25), we now report the results of a complete dose-response experiment in an attempt to reconcile these studies and as a first attempt to determine those specific loci responsible for early opiate-induced effects.

METHOD

Subjects were 28 male, Sprague-Dawley derived rats (Holtzman Co., Madison, WI) weighing 250–300 g. Rats were individually housed with food and water available ad lib. Lights were on between 1900–0700 h.

Chronic polyethylene-silastic catheters were implanted into the right jugular vein after the method of Weeks (41) under Chloropent® anesthesia (2.5 ml/kg) and externalized dorsally to exit between the scapulae. After approximately one week recovery, rats were placed individually into Plexiglas operant chambers within a large sound-attenuated room. Following a 5-min acclimation period, rats received an IV injection of heroin HCl at either 0.0, 0.1, 0.3 or 1.0 mg/kg or naloxone at 1.0 mg/kg or a cocktail of 0.3 mg/kg heroin plus 1.0 mg/kg naloxone. Drugs

TABLE 1

RELATIVE OPTICAL DENSITY DATA (MEAN ± S.E.) FROM EACH OF THE DRUG TREATMENT GROUPS

	Heroin								Naloxone		Cocktail		
Area	1 0.0 mg/kg		2 0.1 mg/kg		3 0.3 mg/kg		4 1.0 mg/kg		5 1.0 mg/kg		6 0.3/1.0 mg/kg		Significance
Mesocorticolimbic													
Amygdala, Basolateral n. (Anterior)	39.6	0.4	40.7	1.0	40.5	0.6	37.7	0.5	40.4	0.1	39.3	1.0	2,3,5>4
Amygdala, Basolateral n. (Posterior)	38.4	0.7	38.7	0.8	38.5	0.9	33.6	0.4	34.1	0.3	39.5	1.8	1,2,3,6>4,5
Amygdala, Central n.	27.5	0.3	29.5	1.4	27.9	0.8	26.3	0.5	29.7	0.4	30.1	1.1	2,6>4
Amygdala, Corticomedial n. (Anterior)	33.0	0.3	33.6	1.2	33.3	0.7	30.9	0.3	35.3	0.2	34.5	1.2	5>4
Amygdala, Corticomedial n. (Posterior)	34.2	0.2	36.3	1.7	36.1	0.6	35.0	1.4	37.2	0.2	37.7	1.4	
Bed Nucleus of Stria Terminalis	32.5	0.8	26.8	1.3	30.2	0.8	28.5	1.0	33.2	0.8	32.9	1.9	1,5,6>2
Claustrum	58.4	0.9	57.9	1.1	57.7	2.4	59.0	0.8	55.5	5.7	52.4	3.9	
Dentate Gyrus	41.2	0.7	41.6	1.6	44.4	1.5	47.2	1.3	42.2	0.6	44.9	1.4	4>1,2,5
Diagonal Band of Broca	55.9	1.3	53.8	0.9	54.8	1.1	56.7	0.4	53.2	3.5	53.9	0.8	
Entorhinal Cortex	46.6	0.5	44.7	2.1	40.9	1.1	37.6	1.3	44.8	0.2	43.0	1.2	1,2,5,6>4;1>3
Hippocampus	29.2	0.3	29.8	1.0	32.1	1.2	32.3	0.6	29.7	0.6	32.0	1.4	
Lateral Septum	38.4	1.6	35.4	0.5	38.4	1.2	38.6	1.1	36.9	0.4	35.4	1.7	
Medial Septum	52.8	0.9	49.3	1.0	50.8	0.7	53.1	1.1	50.8	1.5	48.6	2.3	
Nucleus Accumbens (Anterior)	38.5	1.6	40.4	1.7	39.9	0.5	40.8	1.9	41.5	0.5	40.4	1.5	
Nucleus Accumbens (Posterior)	37.6	2.6	40.4	1.2	40.9	1.6	36.8	1.2	39.4	0.7	38.3	2.3	
Olfactory Tubercle (Anterior)	39.2	2.8	40.6	2.8	39.6	0.3	43.5	1.5	50.1	2.3	42.2	3.5	5>1,2,3,4
Olfactory Tubercle (Posterior)	42.1	1.3	43.9	2.6	40.6	0.8	41.8	0.6	47.6	2.0	45.2	1.4	
Prepyriform Cortex	49.7	0.9	58.0	1.1	57.9	0.7	58.3	0.5	56.9	1.3	57.6	1.6	
Ventral Tegmental Area	42.2	1.6	45.1	3.8	40.9	1.5	44.7	2.0	45.6	2.1	42.9	0.8	
Neocortical													
Cingulate Cortex (Anterior)	55.0	0.3	49.9	1.0	51.6	1.9	47.9	1.1	48.8	1.1	52.5	1.7	1>4
Cingulate Cortex (Posterior)	62.9	1.2	60.9	1.2	60.2	1.0	60.6	0.9	57.7	0.9	61.2	1.0	1>5
Claustrocortex (Anterior)	44.4	0.7	46.9	0.8	48.0	1.1	43.9	0.5	46.1	0.5	43.2	0.6	3>1,4,6;2>6
Claustrocortex (Posterior)	44.5	0.9	46.4	0.9	43.7	0.9	42.7	0.7	45.1	0.6	44.6	2.7	
Frontal Cortex—2° motor cortex (Anterior)	52.5	0.7	50.9	1.9	51.0	1.4	50.9	0.9	48.1	1.9	50.7	1.5	
Frontal Cortex—2° motor cortex (Posterior)	59.5	0.9	58.0	1.1	57.9	0.7	58.3	0.5	56.9	1.3	57.6	1.6	
Medial Prefrontal Cortex	63.3	0.8	62.6	1.7	63.4	0.7	64.6	0.6	60.5	0.7	62.4	0.7	
Thalamus and Hypothalamus													
Anterior Hypothalamus	43.3	0.7	40.8	1.2	41.5	0.6	40.5	1.4	43.7	0.3	42.8	1.9	
Arcuate Nucleus	48.4	5.0	43.3	5.0	39.6	1.5	37.3	3.2	39.6	0.6	41.8	3.1	
Dorsal Medial Hypothalamus	41.4	0.4	37.7	1.8	34.2	0.4	36.0	1.7	42.1	1.0	40.1	1.7	1,5,6>3;5>4
Lateral Dorsal Nucleus Thalamus	61.9	0.7	60.9	3.7	63.7	1.1	70.7	1.3	63.7	1.8	64.1	1.8	4>2,6
Lateral Habenula (Anterior)	65.7	1.4	65.1	1.7	64.6	1.6	68.5	0.8	66.9	1.5	65.1	1.2	
Lateral Habenula (Posterior)	68.9	2.5	64.9	1.7	66.0	0.6	65.9	2.0	65.3	0.5	64.6	0.4	
Lateral Hypothalamus (Anterior)	40.8	0.5	41.2	1.3	38.0	0.3	40.0	0.4	40.4	1.0	40.9	2.3	
Lateral Hypothalamus (Mid)	40.6	0.9	40.2	1.5	40.0	0.6	40.3	0.4	40.7	0.6	40.1	0.5	
Lateral Hypothalamus (Posterior)	41.5	1.8	40.3	0.3	38.5	0.3	40.4	0.5	41.0	1.1	40.0	1.5	
Medial Dorsal Nucleus Thalamus	59.9	1.7	55.9	2.5	54.7	0.8	58.7	0.2	57.2	0.4	60.5	1.0	
Medial Habenula (Anterior)	54.7	2.5	53.5	1.5	54.6	1.2	57.5	2.4	57.1	3.4	59.5	2.1	
Medial Habenula (Posterior)	57.0	4.2	51.2	2.5	55.6	1.9	59.5	2.2	58.1	2.7	58.6	2.5	
Medial Preoptic Area	40.2	1.5	35.7	0.5	35.9	1.3	34.1	1.4	40.0	0.2	36.3	2.2	1,5>4
Paraventricular Nucleus	69.3	0.5	68.8	1.3	68.8	1.6	60.4	1.0	74.5	1.1	69.1	3.8	1,2,3,6,5>4;5>1
Posterior Hypothalamus	44.7	1.2	47.0	1.8	46.4	1.1	48.1	1.1	49.6	1.7	45.8	1.5	
Reuniens Nucleus	57.7	2.7	58.8	1.4	57.1	0.9	57.9	1.2	63.5	1.6	54.8	2.7	5>6
Subthalamus	67.4	0.7	67.4	2.7	64.1	1.5	70.7	1.5	67.3	1.5	68.3	0.9	
Ventral Medial Hypothalamus (Anterior)	35.4	0.6	35.6	1.3	33.9	0.8	35.6	0.8	35.9	0.7	35.2	1.4	
Ventral Medial Hypothalamus (Posterior)	35.1	0.7	31.5	1.4	32.6	2.0	33.6	0.8	35.3	1.0	35.3	2.6	
Ventral Posterior Lateral Thalamus	54.3	0.2	54.7	1.3	54.5	0.8	55.9	0.3	55.9	0.4	56.5	0.2	
Zona Incerta (Anterior)	47.6	1.3	51.6	2.2	50.0	0.5	51.2	0.7	50.8	1.0	49.0	0.5	
Zona Incerta (Posterior)	50.1	0.2	53.8	6.3	53.3	1.0	53.5	0.9	53.1	0.7	52.0	2.0	

were dissolved in saline and injected in a volume of 0.25 ml followed by 0.1 ml saline to flush the catheter. One min later, 160 μCi/kg of the blood flow marker [1-^{14}C] octanoic acid was delivered IV in 0.25 ml saline. Two min later, rats were sacrificed via decapitation, brains rapidly removed, frozen in isopentane (−40°C) and stored at −80°C until sectioned. Frozen serial sections were cut at 16 μm, thaw mounted onto glass slides, dried for 10 min at 50°C on a warming tray and apposed to X-ray film (MR-1; Kodak) for 8–10 weeks.

Autoradiograms were analyzed by computer densitometry us-

TABLE 1 (continued)

Area	Heroin 1 0.0 mg/kg		Heroin 2 0.1 mg/kg		Heroin 3 0.3 mg/kg		Heroin 4 1.0 mg/kg		Naloxone 5 1.0 mg/kg		Cocktail 6 0.3/1.0 mg/kg		Significance
Nigrostriatal System and Related Extrapyramidal Areas													
Caudate Nucleus (Anterior)	37.8	0.6	35.8	1.1	34.5	0.8	35.5	1.2	33.6	0.5	37.3	1.5	
Caudate Nucleus (Mid)	39.0	0.4	39.9	0.4	37.8	0.7	38.3	0.9	37.8	0.2	37.9	1.1	
Caudate Nucleus (Posterior)	40.9	0.3	43.4	0.7	40.8	0.5	41.7	0.8	40.2	0.5	40.9	0.9	2>1,5,6
Endopiriform Nucleus (Anterior)	29.2	2.4	30.7	0.9	31.6	0.8	29.7	1.1	29.4	0.9	29.9	2.6	
Endopiriform Nucleus (Posterior)	28.8	0.9	30.1	1.1	29.0	0.9	27.2	0.5	28.9	1.3	22.3	1.7	
Globus Pallidus	28.3	0.9	28.2	1.2	26.1	0.6	30.6	1.3	28.8	0.4	30.7	0.6	4>3
Substantia Nigra pars reticulata	32.1	0.6	32.6	1.7	31.0	1.0	34.9	0.7	32.7	1.2	30.2	1.4	
Substantia Nigra pars compacta	35.7	0.5	38.8	1.5	35.7	0.2	39.2	0.6	39.2	0.6	35.5	1.6	
Midbrain and Brainstem													
Central Gray	42.4	1.7	39.6	1.2	39.4	1.0	40.9	2.1	43.3	2.0	42.3	1.0	
Dorsal Raphe	55.2	2.5	51.2	1.2	46.9	3.4	55.0	2.6	61.4	0.6	58.5	2.4	4,5,6>3;5>2
White matter	8.9	0.5	7.6	0.7	7.9	0.4	7.5	0.5	7.3	1.0	7.2	0.1	

Brain structures of interest are grouped roughly into functional units. Significance, as determined from analyses of variance and tests for simple effects (Newman-Keuls), are indicated by treatment group codes.

Values are mean ± s.e. represented as a relative optical density score. Group sizes were: gp1 (n=4), gp2 (n=5), gp3 (n=5), gp4 (n=5), gp5 (n=5), gp6 (n=4).

ing an MCID Image Analyzer (Imaging Research, St. Catherines, Ont.). Each defined brain structure was represented by bilateral readings from five separate sections spaced 50 to 80 μm apart. The corresponding thionin-stained sections were used to define structures anatomically. Optical densities (OD) were converted to a relative optical density score (ROD) according to the method of Gallistel et al. (11). Briefly, to obtain an ROD of an area, a frequency histogram is generated consisting of the number of pixels at each of the possible 256 gray scale OD values for the entire digitized brain section. This distribution is then converted to a cumulative frequency histogram by sequentially adding up all the pixels in the histogram. By dividing the new distribution into a percent, the mean OD of the anatomic structure can then be converted to an ROD by its position on the curve. Thus an ROD value of a region represents its density rank relative to the other pixels comprising the entire autoradiogram such that an ROD of 0.6 would mean that the average pixel OD of the structure of interest is darker than 60% of the pixels in that section. This analysis technique has been shown to be more robust and sensitive than a simple gray/white ratio, accounts for the nonlinear relationship between OD and radioactivity, and allows standardization to be made to the brain section from which an area is analyzed (11).

Since the ROD is a percent, an arc sine transform was performed prior to analyses of variance to insure independence of variance (30). Post hoc comparisons were performed with the Student Newman Keuls test. Significance was set at $p<0.05$.

RESULTS

Table 1 depicts the results of the single, acute injection of heroin (0.1, 0.3 or 1.0 mg/kg), naloxone (1.0 mg/kg) or the cocktail injection of naloxone plus heroin on metabolic activity in the rat brain. Data are expressed as the mean relative optical density score (ROD) for each treatment. Sixteen of the 58 structures analyzed were significantly altered by either heroin or naloxone. Heroin-induced increases in octanoate labeling were seen in the caudate nucleus and claustrocortex at the low dose and laterodorsal nucleus of the thalamus and dentate gyrus only at the highest (1.0 mg/kg) dose (Fig. 1). Heroin-induced decreases were seen at the lowest threshold in the bed nucleus of the stria terminalis, after the middle dose in the dorsomedial nucleus of the hypothalamus, dorsal raphe and entorhinal cortex, and only after the highest heroin dose in the cingulate cortex, basolateral nucleus of the amygdala and the medial preoptic and paraventricular nuclei of the hypothalamus (Fig. 2). Naloxone-induced increases in labeling were seen in the olfactory tubercle and hypothalamic paraventricular nucleus, while decreases were seen in the posterior cingulate cortex and basolateral nucleus of the amygdala (Fig. 3).

DISCUSSION

The use of labelled octanoate (OCTO) as a metabolic marker was first introduced by Rowley and Collins (32). This 8-carbon free-fatty acid is rapidly cleared from blood and ultimately β-oxidized to produce a labelled pool of glutamate. As it is known that glutamate synthetase is found predominantly within protoplasmic astrocytes (28), it is likely that OCTO accumulation is due to its entry into astrocytes lining cerebral capillaries and thus reflecting changes in blood flow. The conversion to glutamate requires about 6 min and therefore sacrifice times earlier than that will reflect trapped label in these glial cells. The sequence by which changes in astrocytes might reflect neuronal activity is suggested by the observation that increases in extracellular potassium accumulation after neuronal activation leads to an increase in intracellular astrocyte pH, thus suggesting a mechanism for local alterations in CBF (4). Despite the inability at present to directly quantify blood flow via OCTO accumulation or metabolism, the relative regional accumulation of tracer appears to provide a useful and accurate indicator of rapid functional activity in brain (32, 38, 39).

It should be pointed out that a potential flaw in using a relative (normalized) measure of octanoate labeling might exist if the treatment had a generalized effect on cerebral blood flow. If, for example, heroin globally decreased blood flow, then areas showing an increase using the ROD method might be unaffected when using full quantification. To test for this possibility,

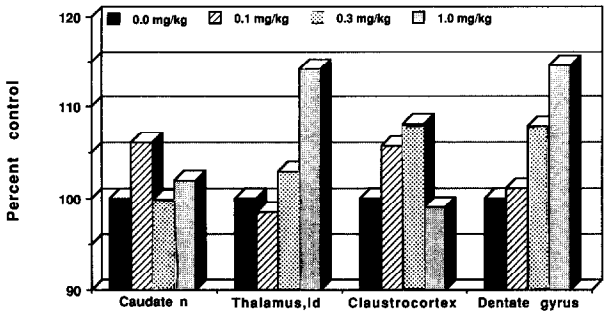

FIG. 1. Ability of heroin to increase local blood flow as measured by OCTO labeling in specific rat brain nuclei. Data presented are the mean relative optical density (ROD) for each treatment group expressed as a percent of the saline control group. Significant treatment alterations include increases after 0.1 mg/kg in the caudate nucleus; 0.1 and 0.3 mg/kg in the claustrocortex and 1.0 mg/kg in the laterodorsal nucleus of the thalamus, and dentate gyrus.

we calculated an overall mean OD value for each of the six treatment groups by averaging the data across all 58 areas of interest. An analysis of variance on these data revealed no significant differences between groups, $F(5,27) = 1.144$, $p<0.37$. It thus appears that global drug effects were not responsible for the alterations in regional blood flow reported herein.

Results from this experiment utilizing octanoate appear to bridge those obtained previously using the blood flow marker iodoantipyrene (IAP) and the metabolic marker 2-deoxyglucose (2-DG). Opiate agonists are generally regarded as CNS depressants with such measures as behavioral and neuronal activity and cerebral metabolism generally becoming depressed after administration (25). In contrast, many regions of the brain have been demonstrated to be activated by opioids with consequent increases in cerebral blood flow. The four regions which were activated by heroin in this experiment—the caudate nucleus, laterodorsal thalamus, claustrocortex and dentate gyrus—also demonstrated an increase in regional cerebral blood flow (rCBF) after 0.2 mg/kg heroin in our previous IAP study (37). Blum et al. (2) observed an increase in rCBF in basal ganglia, cerebellum, pons, parietal and frontal cortex (but not hippocampus, occipital cortex or medulla) in miniature swine after infusion of methionine enkephalin, although as the time course was rather long in their study, the authors note that such alterations may either have reflected metabolic consequences of the peptide or a direct action on the cerebral vasculature. Increases in blood flow have also been reported in rabbit caudate nucleus, thalamus, colliculi, pons and cerebellum after 2 mg/kg IV morphine (20).

In contrast, while the decreases in octanoate labeling reported here do not generally reflect these prior results, they do generally agree with both our previous octanoate study (39) and several 2-DG reports of decreases in lcgu (1, 9, 33). The latter generally report these decreases in such areas as the striatum, basal ganglia, thalamus and substantia nigra. In contrast, a handful of studies have reported increases in lcgu after morphine. Ito et al. (18) observed increased activity only in the substantia nigra pars reticulata and mammillary body after SQ morphine. In addition, while Glick et al. (13) found a selective increase in striatum in rats self-administering morphine, since passive morphine delivery in yoked controls did not alter metabolism in this area, it is likely that other behavioral events were reflected in their reported increase in lcgu.

We found four regions altered by naloxone—the cingulate and basolateral nucleus of the amygdala displayed decreased blood flow, while the olfactory tubercle and paraventricular nu-

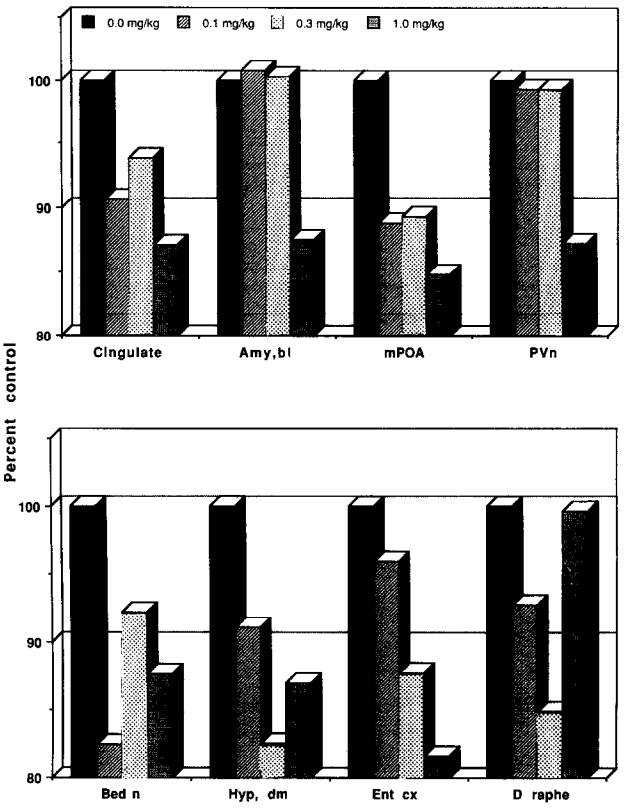

FIG. 2. Heroin-induced decreases in blood flow. Data are presented as in Fig. 1. Areas decreased by the highest heroin dose (1.0 mg/kg) include the cingulate cortex, medial preoptic area, paraventricular nucleus of the hypothalamus, and basolateral nucleus of the amygdala, the bed nucleus of the stria terminalis was depressed only by the lowest dose (0.1 mg/kg), the dorsomedial of the hypothalamus and dorsal raphe at the mid (0.3 mg/kg) dose while the entorhinal cortex was depressed by the mid and high dose.

cleus had increased OCTO labeling. Other studies have reported a naloxone-induced decrease in CBF in entorhinal cortex in the resting state (37), and increases after anoxic challenge (29), and after withdrawal from chronic naltrexone in cortex, cerebellum, thalamus and pons (but not hippocampus, hypothalamus or medulla) (3). Acute naltrexone has also been reported to decrease CBF (14). In contrast, no naloxone-induced alterations in glucose uptake have been reported for any forebrain structure examined (12), nor any alterations in lcgu from a survey of 58 brain regions in the rat (10). However, naloxone-induced decreases were seen in 18 brainstem nuclei (16), and a decrease in lcgu in the striatum (but not 6 other regions) have been reported during chronic naltrexone administration (7).

The significance of naloxone-induced alterations in blood flow is not clear at this time. Such alterations would seem to imply that cells in these regions have a significantly higher endogenous opiatergic tone with appreciable intrinsic activity which is then reversed by naloxone. It is curious, however, if there is such an endogenous tone, that the administration of naloxone generally results in such unremarkable behavioral alterations (25). It may be that the behavioral assays utilized are not sufficiently sensitive or do not measure appropriate parameter to reflect these putative tonic, cellular modulatory effects of opioids.

The discrepancies between the blood flow and glucose metabolism studies reviewed above can likely be accounted for by methodologic considerations. One possibility is that cells which

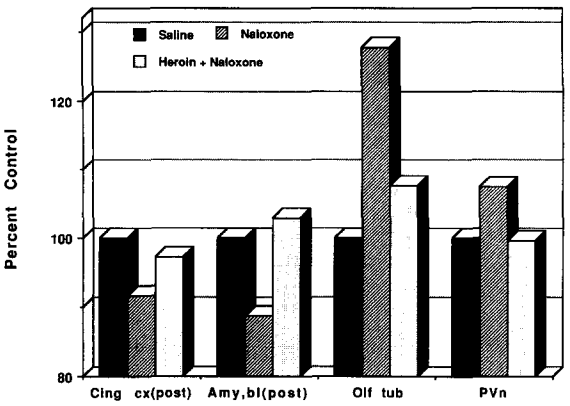

FIG. 3. Alterations in blood flow as a consequence of 1.0 mg/kg naloxone administration. Data presented as in Fig. 1. Increases in flow were seen in the olfactory tubercle and paraventricular nucleus of the hypothalamus. Decreases in flow were found in the posterior cingulate cortex and basolateral nucleus of the amygdala.

are activated by opiates are too few and widely dispersed within structures to be resolved by the relatively coarse grain resolution of metabolic autoradiographic mapping and/or are not of sufficient magnitude to influence the large amount of either nonreactive or inhibited tissue which surrounds these zones (21). While this is certainly a possibility, our previous report of increases in rCBF (37) and that of Smith and colleagues (34) of increases in regional neurotransmitter turnover levels would argue against this suggestion. Probably the largest contributors to the disparate results are the time windows employed, the markers used and methodologic constraints these different techniques engender and the routes of drug administration employed across these studies. The pharmacodynamics of heroin indicate that it is very time and dose dependent such that after high doses neuronal and behavioral depression are seen immediately and excitation somewhat later, while excitation appears immediately after low doses (25). It is thus difficult to compare results between studies when label latencies after drug have varied from 0 to 60 min, sacrifice times after label from 1 min to 2 h, and route of drug delivery either IV, SQ or IP. What is clear from these studies, however, is that issues of pharmacodynamics must be considered when designing and interpreting metabolic mapping studies of drug effects. For example, after the administration of high doses of IP cocaine, London et al. (22) observed cocaine-induced activation mainly within extrapyramidal structures, while Porrino et al. (31), utilizing lower doses of IV cocaine, reported predominantly activation of mesolimbic and mesocortical structures. Thus, apparently due to differences in dose and drug routes, the mix of structures activated in these studies was quite distinct. Others have also demonstrated that route of administration is a significant variable in the establishment of conditioned place preference in rats (27,36).

Most of the structures inhibited by heroin in this study are constituents of, or receive projections from limbic structures. Many hypothalamic nuclei were inhibited including the paraventricular nuclei which, with its outputs to the median eminence and posterior pituitary, may reflect some of the inhibitory influence of opiates on neuroendocrine functions (25).

Four regions were activated by heroin in this study; the dentate, caudate, claustrocortex and laterodorsal nucleus of the thalamus. That opiates can activate neuronal elements is not a unique observation. Electrophysiologic experiments have revealed that cells within the ventral tegmentum, substantia nigra, periaqueductal gray, amygdala and caudate can all be excited by opiate administration (5, 6, 15, 19, 26). The lack of complete concurrence between these sets of data may be partially reconciled by differences in measurements—electrophysiology vs. rCBF—each having its own time, sampling and sensitivity constraints. The advantages of global autoradiographic techniques must be considered in light of the excellent temporal resolution and real time analysis of single cell recording following either systemic or iontophoretic drug application.

At least three of the regions which we found to be excited by heroin are strongly interconnected—the dentate and laterodorsal thalamus communicates with the hippocampus and entorhinal cortex, while the claustrocortex interconnects with the caudate and entorhinal cortex. Smith and colleagues (34) have proposed two putative reward circuits based on their neurotransmitter turnover experiments: one from hippocampus to nucleus accumbens to amygdala to entorhinal cortex and back to hippocampus and a second from frontal cortex to striatum to globus pallidus back to frontal cortex. Taken together with additional pharmacologic and behavioral manipulations, the overall goal of this type of metabolic mapping research is to be able to ultimately hypothesize putative CNS circuits mediating various aspects of the pharmacologic profile of opiate agents.

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REFERENCES

1. Beck, T.; Wenzel, J.; Kuschinsky, K.; Krieglstein, J. Morphine-induced alterations of local cerebral glucose utilization in the basal ganglia of rats. Brain Res. 497:205–213; 1989.
2. Blum, K.; Gaskill, H.; DeLallo, L.; Briggs, A. H.; Hall, W. Methionine enkephalin as a possible neuromodulator of regional cerebral blood flow. Experientia 41:932–933; 1985.
3. Buchweitz, E.; Weiss, H. R. Effect of withdrawal from chronic naltrexone on regional cerebral oxygen consumption in the cat. Brain Res. 397:308–314; 1986.
4. Chesler, M.; Kraig, R. P. Intracellular pH of astrocytes rises rapidly with cortical stimulation. Soc. Neurosci. Abstr. 13:256; 1987.
5. Chou, D. T.; Wang, S. C. Unit activity of amygdala and hippocampal neurons: effects of morphine and benzodiazepines. Brain Res. 126:427–440; 1977.
6. Dafny, N.; Brown, M.; Burks, T. F.; Rigor, B. M. Patterns of unit responses to incremental doses of morphine in central gray, reticular formation, medial thalamus, caudate nucleus, hypothalamus, septum and hippocampus in unanesthetized rats. Neuropharmacology 18:489–496; 1979.
7. Dow-Edwards, D.; Milhorat, T. H.; Freed, L. A.; Gintzler, G. R. Regional brain glucose utilization during and following chronic naltrexone administration: preliminary observations in the rat brain. Life Sci. 44:571–577; 1989.
8. Duggan, A. W.; North, R. A. Electrophysiology of opioids. Pharmacol. Rev. 35:219–281; 1984.
9. Fanelli, R. J.; Szikszay, M.; Jasinski, D. R.; London, E. D. Differential effects of mu and kappa opioid analgesics on cerebral glucose utilization in the rat. Brain Res. 422:257–266; 1987.
10. Fanelli, R. J.; Walovitch, R. C.; Jasinski, D. R.; London, E. D. Naloxone fails to alter local cerebral glucose utilization in the rat. Pharmacol. Biochem. Behav. 31:481–485; 1988.
11. Gallistel, C. R.; Piner, C. T.; Allen, T. O.; Adler, N. T.; Yadin, E.; Negin, M. Computer-assisted analysis of 2-DG autoradiograms. Neurosci. Biobehav. Rev. 6:409–420; 1982.
12. Geary, W. A., II; Wooten, G. F. Dose effects of naloxone on fixed morphine dependence: Simultaneous behavioral and 2-deoxyglucose

study in the rat. Brain Res. 332:69–78; 1985.
13. Glick, S. D.; Cox, R. D.; Meiback, R. C. Selective effect of reinforcing doses of morphine in striatum. Brain Res. 190:298–300; 1980.
14. Grandison, L.; Buchweitz, E.; Weiss, H. R. Effect of naltrexone on regional brain oxygen consumption in the cat. Brain Res. 223: 369–379; 1982.
15. Gysling, K.; Wang, R. Y. Morphine-induced activation of A10 dopamine neurons in the rat. Brain Res. 277:119–127; 1983.
16. Hayashi, T.; Nakamura, K. Localized effects of naloxone on local cerebral glucose utilization in rat cerebral nuclei with met-enkephalinergic neurons. Jpn. J. Pharmacol. 36:339–348; 1984.
17. Hiesiger, E. M.; Voorhies, R. M.; Lipschutz, L.; Basler, G.; Shapiro, W. R.; Pasternak, G. W. The effects of morphine on glucose metabolism ([^{14}C]2-deoxyglucose) in rat brain as measured by quantitative autoradiography. Soc. Neurosci. Abstr. 1:138; 1983.
18. Ito, M.; Suda, S.; Namba, H.; Sokoloff, L.; Kennedy, C. Effects of acute morphine administration on local cerebral glucose utilization in the rat. J. Cereb. Blood Flow Metab. 3:S574–S575; 1983.
19. Iwatsubo, K.; Clouet, D. H. Effects of morphine and haloperidol on the electrical activity of rat nigrostriatal neurons. J. Pharmacol. Exp. Ther. 202:429–436; 1977.
20. Koskihen, L. O.; Bill, A. Regional cerebral, ocular and peripheral vascular effects of naloxone and morphine in unanesthetized rabbits. Acta Physiol. Scand. 119:235–241; 1983.
21. Levy, R. M.; Fields, H. L.; Stryker, M. P.; Heinricher, M. M. The effect of analgesic doses of morphine on regional cerebral glucose metabolism in pain-related structures. Brain Res. 368:170–173; 1986.
22. London, E. D.; Wilkerson, G.; Goldberg, S. R.; Risner, M. E. Effects of l-cocaine on local cerebral glucose utilization in the rat. Neurosci. Lett. 68:73–78; 1986.
23. Mansour, A.; Khachaturian, H.; Lewis, M. E.; Akil, H.; Watson, S. J. Autoradiographic differentiation of mu, delta and kappa opioid receptors in the rat forebrain and midbrain. J. Neurosci. 7:2445–2464; 1987.
24. Mansour, A.; Khachaturian, H.; Lewis, M. E.; Akil, H.; Watson, S. J. Anatomy of CNS opioid receptors. Trends Neurosci. 11:308–314; 1988.
25. Martin, W. R. Pharmacology of opioids. Pharmacol. Rev. 35:282–323; 1984.
26. Matthews, R. T.; German, D. C. Electrophysiological evidence for excitation of rat ventral tegmental area dopamine neurons by morphine. Neuroscience 11:617–625; 1984.
27. Nomikos, G. G.; Spyraki, C. Cocaine-induced place preference conditioning: Importance of route of administration and other procedural variables. Psychopharmacology (Berlin) 94:119–125; 1988.
28. Norenberg, M. D.; Martinez-Hernandez, A. Fine structural localization of glutamine synthetase in astrocytes of rat brain. Brain Res. 161:303–310; 1979.
29. Phyllis, J. W.; DeLong, R. E.; Towner, J. K. Naloxone enhances cerebral reactive hyperemia in the rat. Neurosurgery 17:596–599; 1985.
30. Pollard, J. H. A handbook of numerical and statistical techniques. Cambridge: Cambridge University Press; 1977.
31. Porrino, L. J.; Domer, F. R.; Crane, A. M.; Sokoloff, L. Selective alterations in cerebral metabolism within the mesocorticolimbic dopaminergic system produced by acute cocaine administration in rats. Neuropsychopharmacology 1:109–118; 1988.
32. Rowley, H.; Collins, R. C. [1-^{14}C]octanoate: a fast functional marker of brain activity. Brain Res. 335:326–329; 1985.
33. Sakurada, O.; Shirohara, M.; Klee, W. A.; Kennedy, C.; Sokoloff, L. Local cerebral glucose utilization following acute or chronic morphine administration and withdrawal. Soc. Neurosci. Abstr. 2:613; 1976.
34. Smith, J. E.; Lane, J. D. Brain neurotransmitter turnover correlated with morphine self administration. In: Smith, J. E.; Lane, J. D., eds. The neurobiology of opiate reward processes. Amsterdam: Elsevier; 1983:361–402.
35. Smith, J. E.; Lane, J. D., eds. The neurobiology of opiate reward processes. Amsterdam: Elsevier; 1983.
36. Spyraki, C.; Nomikos, G. G.; Varonos, D. D. Intravenous cocaine-induced place preference: Attenuation by haloperidol. Behav. Brain Res. 26:57–62; 1982.
37. Trusk, T. C.; Stein, E. A. Effects of intravenous heroin and naloxone on regional cerebral blood flow in the conscious rat. Brain Res. 406:238–245; 1987.
38. Trusk, T. C.; Stein, E. A. Effect of heroin-conditioned auditory stimuli on cerebral functional activity in rats. Pharmacol. Biochem. Behav. 30:983–993; 1988.
39. Trusk, T. C.; Stein, E. A. Effects of heroin and cocaine on brain activity in rats using [1-^{14}C] octanoate as a fast functional tracer. Brain Res. 438:61–66; 1988.
40. Watson, S. J.; Trujillo, K. A.; Herman, J. P.; Akil, H. Neuroanatomical and neurochemical substrates of drug seeking behavior: overview and future directions. In: Goldstein, A., ed. Molecular and cellular aspects of the drug addictions. New York: Springer Verlag; 1989:29–91.
41. Weeks, J. R. Experimental morphine addiction: Methods for automatic intravenous injections in unrestrained rats. Science 138:143–144; 1962.