MOLECULAR PHARMACOLOGY, 14, 69–76

[^{3}H]Opiate Binding: Anomalous Properties in Kidney and Liver Membranes

RABI SIMANTOV,[1] STEVEN R. CHILDERS, AND SOLOMON H. SNYDER[2]

Departments of Pharmacology and Experimental Therapeutics and of Psychiatry and the Behavioral Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

(Received February 21, 1977)
(Accepted September 6, 1977)

SUMMARY

SIMANTOV, RABI, CHILDERS, STEVEN R. & SNYDER, SOLOMON H. (1978) [^{3}H]Opiate binding: anomalous properties in kidney and liver membranes. *Mol. Pharmacol.*, 14, 69–76.

[^{3}H]Naloxone and [^{3}H]dihydromorphine bind in a saturable fashion and with high affinity to membrane preparations of guinea pig kidney and liver. Binding in guinea pig kidney displays "reversed" stereospecificity, with pharmacologically inactive dextrallorphan being more potent than the known pharmacologically active levallorphan. Opiate agonists tend to be more potent than their corresponding antagonists in competing for [^{3}H]opiate binding in guinea pig kidney. Unlike brain opiate receptors, in which sodium and manganese selectively decrease and increase, respectively, the binding of [^{3}H]opiate agonists, these ions have no selective effect on the binding of [^{3}H]opiates in guinea pig kidney and liver. The opioid peptides Met-enkephalin and β-endorphin and the opiates etorphine and diprenorphine, which have very high affinity for brain opiate receptors, have negligible effects on [^{3}H]opiate binding in guinea pig kidney.

INTRODUCTION

Opiates affect numerous functions throughout the body. Most of these influences are thought to involve initial actions upon the brain and/or pituitary, while opiates presumably exert some direct actions in the intestines. To determine whether there might be hitherto unrecognized peripheral actions of opiates, we screened a variety of animal organs for saturable [^{3}H]opiate binding. We now report the characteristics of saturable [^{3}H]opiate binding to kidney and liver membranes.

This work was supported by Grant DA-00266 from the United States Public Health Service.

[1] Present address, Department of Genetics, The Weizmann Institute of Science, Rehovot, Israel.

[2] Recipient of Research Scientist Career Development Award MH-33128 from the United States Public Health Service.

MATERIALS AND METHODS

Opiate receptor binding assay. Membranes from brain (1), kidney, liver, or other tissue were prepared as follows. The tissue was removed immediately after decapitation, placed in 40 volumes of ice-cold 50 mM Tris-HCl buffer, pH 7.7, at 25°, homogenized with a Brinkmann Polytron PT-10 (setting 5, 20 sec), and centrifuged at 4° for 10 min at 49,000 × g. The pellets were resuspended in Tris buffer (about 10 mg/ml), incubated for 40 min at 37°, centrifuged for 10 min, resuspended in the same buffer (10 mg of original wet tissue per milliliter), and assayed for binding. Binding of [^{3}H]enkephalin was measured at 25° with 1.9 ml of membrane suspen-

0026-895X/78/0141-0069$02.00/0
Copyright © 1978 by Academic Press, Inc.
All rights of reproduction in any form reserved.

sion. After 40 min of incubation the samples were immediately filtered and the radioactivity was monitored as described (1). Saturable binding was defined as the difference between binding in the presence of 1 μM levallorphan and that in its absence. All values referred to as "binding" represent saturable binding unless otherwise indicated. Values are expressed as the means of three replications, which varied less than 15%.

Whenever labeled or unlabeled enkephalin was included in the experiment, 50 μg/ml of bacitracin were included to inhibit enkephalin destruction. This concentration of bacitracin, which was sufficient to protect against enkephalin degradation in brain homogenates (2), was not as effective in kidney preparations. After 40 min of incubation at 25° of 0.1 μM Met-enkephalin in the kidney membrane preparation with bacitracin (50 μg/ml), only 33% of enkephalin activity remained, as assayed by inhibition of [^{3}H]naloxone binding. Without bacitracin, no enkephalin activity remained. However, the synthetic analogue 2-D-Ala-Met-enkephalin was completely stable under these conditions, since no activity was lost after 40 min of incubation with kidney preparations. Therefore 2-D-Ala-Met-enkephalin was included in binding studies of enkephalin in kidney.

Materials. [^{3}H]Naloxone (20 Ci/mmole), [^{3}H]dihydromorphine (45 Ci/mmole), [^{3}H]morphine (12.5 Ci/mmole), [^{3}H]Met-enkephalin (17.4 or 20 Ci/mmole), [^{3}H]Leu-enkephalin (33.4 Ci/mmole), [^{3}H]levallorphan (7.5 Ci/mmole), [^{3}H]levorphanol (5.4 Ci/mmole), [^{3}H]etorphine (30 Ci/mmole), and [^{3}H]diprenorphine (13 Ci/mmole) were purchased from New England Nuclear. [^{3}H]2-D-Ala-enkephalin (25 Ci/mmole) was obtained from Amersham/Searle. Synthetic Met- and Leu-enkephalins, built by fragment condensation methods (3), were generously donated by Drs. D. Hauser and F. Cardinaux, Sandoz, Basel. Synthetic β-endorphin was a generous gift from Professor C. H. Li, University of California. α-Endorphin was purchased from Peninsula Laboratories. Other opiate sources were Endo (naloxone, oxymorphone), Roche (levallorphan, levorphanol), American Cyanamid (etorphine, diprenorphine), Winthrop (pentazocine, cyclazocine, metazocine), and Lilly [(+)- and (−)-propoxyphene, methadone]. The sample of 2-D-Ala-Met-enkephalin was generously donated by Drs. R. Miller and P. Cuatrecasas, Wellcome Research Laboratories, Research Triangle Park, N.C.

RESULTS

Organ distribution of [^{3}H]opiate binding. Saturable binding of [^{3}H]naloxone and [^{3}H]dihydromorphine, measured as the difference between binding in the absence and presence of 1.0 μM levallorphan, can be detected in substantial amounts in guinea pig brain, kidney, liver, and small intestine (Table 1). Much smaller amounts of both [^{3}H]naloxone and [^{3}H]dihydromorphine are bound in testes, fat cells, and lung. Within guinea pig kidney, binding of [^{3}H]naloxone and [^{3}H]dihydromorphine is about twice as great in the cortex as in the medulla (Table 1). In rat tissues, considerable binding of the two [^{3}H]opiates is present in brain, with only a very small amount of binding apparent in kidney and liver.

To determine whether [^{3}H]naloxone binding to kidney membranes is restricted to the guinea pig, we examined binding of three concentrations of [^{3}H]naloxone to kidney membranes of rabbit, rat, calf, and guinea pig (Table 2). Substantial [^{3}H]naloxone binding is apparent in rabbit kidney as well as in the guinea pig. With increasing amounts of [^{3}H]naloxone, binding can be reliably detected in rat and calf kidney. The concentrations of [^{3}H]naloxone employed in these experiments were too low to permit estimates of the saturability of binding in the various species.

[^{3}H]Naloxone binding is saturable in guinea pig liver, brain, and kidney. In all three tissues binding attains maximal levels between 5 and 10 nM. Half-maximal binding in the three tissues occurs between 2 and 3 nM. The maximal levels of binding are greatest in the kidney, being 1.4 times maximal binding in brain and 2.6 times comparable values in the liver (Fig. 1).

Influence of drugs and peptides on

TABLE 1

[^{3}H]Opiate binding to guinea pig and rat membrane preparations from various organs

Organs were dissected immediately after decapitation, and membranes were prepared and assayed by the standard binding procedure at 25° with 3.8 nM [^{3}H]naloxone or 2.0 nM [^{3}H]dihydromorphine and no added ions. Data shown are from a typical experiment performed in triplicate. Each experiment was replicated two to four times, and values varied less than 25%.

Organ	Guinea pig				Rat			
	[^{3}H]Naloxone		[^{3}H]Dihydromorphine		[^{3}H]Naloxone		[^{3}H]Dihydromorphine	
	Total	Saturable	Total	Saturable	Total	Saturable	Total	Saturable
	pmoles bound/g tissue							
Brain	7.95	4.80	2.84	1.44	12.2	7.78	7.39	4.60
Whole kidney	15.5	10.3	12.2	6.43	1.34	0.14	0.40	0.03
Kidney cortex	12.4	7.45	5.79	3.08				
Kidney medulla	5.01	2.85	2.96	1.68				
Liver	8.01	4.45	6.49	3.52	1.61	0.20	0.30	0.09
Heart	1.01	0.22	2.34	0.01	1.51	0.08	0.22	0.02
Lung	2.98	0.20	2.75	0.14	0.52	0.08	0.43	0.08
Stomach	0.48	0.04	0.99	0.04	0.58	0.02	0.20	0.03
Small intestine	3.95	1.87	3.04	1.52	0.88	0.06	0.35	0.03
Large intestine	2.18	0.06	0.88	0.00	1.02	0.02	0.18	0.00
Striated muscle	2.58	0.62	2.10	0.00	1.34	0.04	0.34	0.06
Fat cells	1.90	0.33	2.27	0.16	0.44	0.08	0.10	0.00
Pancreas	0.52	0.07	0.52	0.00	0.25	0.04	0.20	0.01
Testes	2.45	0.37	2.62	0.23	0.68	0.17	0.43	0.04

TABLE 2

Species variations in [^{3}H]naloxone binding to kidney membranes

The experiment was performed as described in Table 1, with no ions added. The calf kidneys were frozen 20–30 min after death and maintained at −70° for 2 hr before the membranes were prepared. Kidneys from the other species were taken immediately after decapitation. Data are from typical experiments, each of which was replicated two or three times. Values varied less than 25%.

[^{3}H]Naloxone added	Guinea pig		Rabbit		Rat		Calf	
	Total	Saturable	Total	Saturable	Total	Saturable	Total	Saturable
nM	*pmoles bound/g tissue*							
1.75	5.97	2.43	5.62	2.95	0.81	0.13	0.66	0.20
3.5	10.5	5.94	11.4	6.29	2.85	0.36	2.46	0.23
5.0	16.7	8.92	15.6	7.82	5.59	0.55	4.84	0.58

[^{3}H]opiate binding in brain, kidney, and liver. Guinea pig brain and kidney display marked differences in drug specificity (Table 3 and Fig. 2). The most striking of these is a reversed stereospecificity for certain drugs, observed in the kidney. Whereas the pharmacologically active isomer levallorphan is more than 1000 times as potent as the pharmacologically inactive dextrallorphan in reducing [^{3}H]-naloxone binding in the guinea pig brain, the stereospecificity is reversed in guinea pig kidney, with dextrallorphan being about 10 times more potent than levallorphan. While the pharmacologically active *d*-propoxyphene is 30 times more potent than *l*-propoxyphene in brain, the two isomers have about the same affinities for [^{3}H]naloxone binding sites in kidney. Similarly, levorphanol is 8000 times more potent than dextrorphan in the brain, but the two drugs are equipotent in the kidney (Table 3).

In an earlier study (4) we observed binding of [^{3}H]naloxone to guinea pig kidney homogenates which was inhibited with

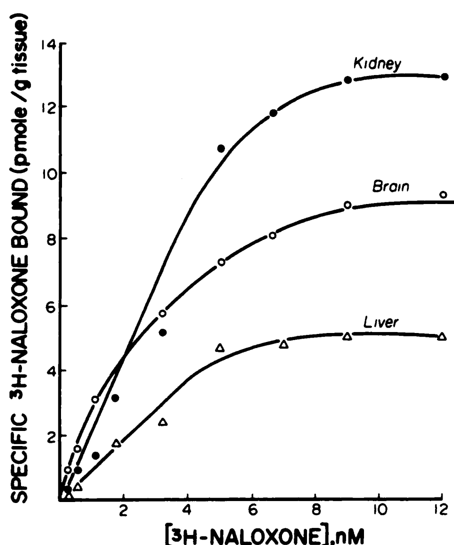

FIG. 1. *Saturation of [³H]naloxone binding to guinea pig brain, kidney, and liver membranes*

The standard binding assay was performed at 25° in a 2-ml volume with 1.9 ml of tissue diluted 1:300 (w/v) (about 3.3 mg of tissue, wet weight, per milliliter). The specific binding was calculated from the total binding after subtracting nonspecific binding obtained in the presence of 1 μM levallorphan. The experiment was replicated twice.

TABLE 3

Stereospecificity of [³H]naloxone binding to guinea pig kidney membranes

[³H]Naloxone (1.5 nM) binding was assayed in the presence of eight to eleven (0.30 nM–10 μM) concentrations of the indicated drugs. Values are the means of two to six determinations and varied less than 25%. IC_{50} is the concentration that inhibits [³H]naloxone binding 50%.

Opiate	IC_{50}		Kidney to brain ratio
	Brain	Kidney	
	nM	*nM*	
Dextrallorphan	4,000	15	0.004
Levallorphan	0.7	150	214
Dextrorphan	4,000	10	0.0025
Levorphanol	0.5	10	20
(−)-Propoxyphene	10,000	1,500	0.15
(+)-Propoxyphene	300	2,000	6.7

greater potency by levorphanol than by dextrorphan, observations confirmed by Musacchio.[3] To account for the apparent discrepancy between the earlier observations and the present findings, we compared the potencies of levorphanol, dextrorphan, dextrallorphan, and levallorphan in competing for [³H]naloxone binding to homogenates and washed membrane preparations of guinea pig kidney with and without prior incubation (Table 4). As reported previously, in whole homogenates levorphanol is indeed more potent than dextrorphan in competing for [³H]naloxone binding. However, the two isomers differ only by a factor of 8, in contrast to the 10,000-fold difference in potency in competing for binding in brain membranes. The same difference in potencies of the two drugs is apparent in washed membrane preparations. However, after prior incubation of the membranes, levorphanol and dextrorphan become equipotent inhibitors of [³H]naloxone binding. The primary effect of prior incubation appears to be enhancement of the potency of

[3] J. Musacchio, personal communication.

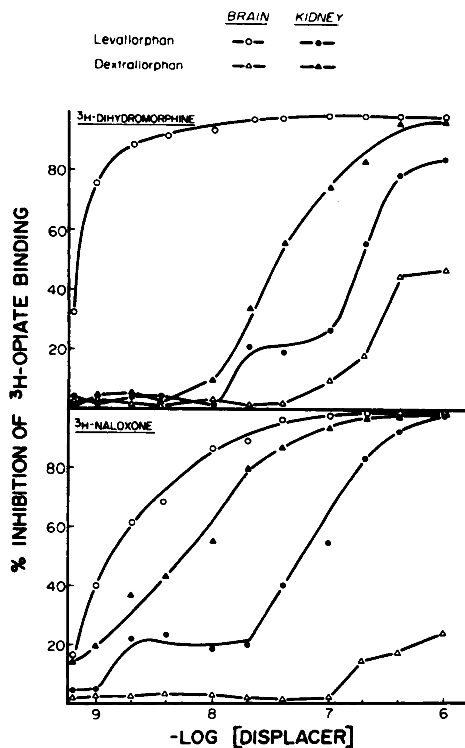

FIG. 2. *Reverse stereospecificity of [³H]naloxone and [³H]dihydromorphine binding to guinea pig kidney membranes*

The experiment was performed with 1.5 nM [³H]naloxone or 1.0 nM [³H]dihydromorphine in the standard binding assay and was replicated twice.

TABLE 4

Effect of prior incubation on stereospecificity of [^{3}H]naloxone binding to guinea pig kidney membranes

[^{3}H]Naloxone (1.5 nM) binding was assayed on kidney tissue either immediately after homogenization, after centrifugation at 49,000 × *g* for 10 min and resuspension in the original volume of 50 mM Tris, pH 7.7, or after centrifugation followed by incubation at 37° for 45 min and a second centrifugation and resuspension. Assays were conducted in the presence of eight (0.3 nM–10 μM) concentrations of the indicated drugs. Values are from a typical experiment, which was replicated three times, and varied less than 30%.

Tissue treatment	IC_{50}			
	Levorphanol	Dextrorphan	Levallorphan	Dextrallorphan
	nM	*nM*	*nM*	*nM*
Homogenate	56	380	140	11
Membranes	27	540	110	18
Membranes + prior incubation	18	17	51	5.2

dextrorphan with little change in the influence of levorphanol.

The "reversed" stereospecificity of levallorphan and dextrallorphan is observed in homogenates, as well as in membranes, with or without prior incubation. Prior incubation appears to enhance somewhat the potencies of both levallorphan and dextrallorphan. Thus guinea pig kidney preparations differ considerably in their interactions with the levorphanol-dextrorphan and levallorphan-dextrallorphan pairs of drugs.

Another striking difference between kidney and brain lies in the failure of the opioid peptides Met-enkephalin, its 2-D-alanine analogue, and β-endorphin to affect [^{3}H]naloxone binding in guinea pig kidney in concentrations as high as 1 μM. By contrast, these agents produce 50% inhibition of [^{3}H]naloxone binding by brain membranes at 8–15 nM. The failure of Met-enkephalin and β-endorphin to affect [^{3}H]naloxone binding is also observed in rabbit kidney (Table 5). The absence of displacement by enkephalin is not caused by increased degradation of enkephalin in kidney, because the 2-D-alanine analogue of enkephalin, which is not significantly degraded in either kidney or brain during standard incubation conditions, is also inactive. Certain other opiates, such as etorphine and diprenorphine, have much less affinity for kidney than brain binding sites. For other drugs, guinea pig kidney

TABLE 5

Displacement of [^{3}H]naloxone binding to guinea pig brain and kidney membranes by opiates and opioid peptides

The following compounds at 5 μM reduced [^{3}H]naloxone binding less than 15%: angiotensin II, glucagon, neurotensin, vasopressin (AVP), substance P, vasotocin, (AVT)m oxytocin, D- or L-epinephrine, D- or L-norepinephrine, D- or L-isoproterenol, D- or L-phenylephrine, dopamine, catechol, ergotamine, ergokryptine, γ-aminobutyric acid, bicuculline, taurine, proline, β-alanine, glycine, glutamic acid, tyrosine, phenylalanine, oxotremorine, probenecid, ouabain, acetylsalicyclic acid, penicillin G, sulfadiazine, benzoic acid, uric acid, hippuric acid, pyruvic acid, lactic acid, succinic acid, citric acid, maleic acid, nicotinamide, tyramine, hexamethonium, and choline. IC_{50} is the concentration of the agent which reduced [^{3}H]naloxone binding by 50%. The experiment was performed as described in Table 3 and MATERIALS AND METHODS. Values are the means of two to six determinations and varied less than 30%.

Displacer	IC_{50}		Kidney to brain ratio
	Brain	Kidney	
	nM	*nM*	
Naloxone	3	60	20
Oxymorphone	4	20	5
Nalorphine	4	600	150
Morphine	10	30	3
Levallorphan	0.7	150	214
Levorphanol	1.5	10	6.7
Naltrexone	0.8	60	75
Dihydromorphine	7	150	21
Cyclazocine	0.5	4	8
Pentazocine	15	100	6.7
Metazocine	30	150	5
Methadone	6	500	83
Etorphine	0.5	700	1400
Diprenorphine	0.8	>1000	>1250
Met-enkephalin	8	>1000	>125
2-D-Ala-Met-enkephalin	15	>1000	>67
β-Endorphin	4	>1000	>250
Haloperidol	6000	500	0.08
Benperidol	500	150	0.3
Pimozide	200	50	0.25

displays affinity in the nanomolar range, although for most drugs affinity is somewhat less in kidney than in brain (Table 5). The only exception consists of the pharmacologically inactive isomers dextrallorphan, dextrorphan, and *l*-propoxyphene, which are substantially more potent in kidney than in brain.

Since these assays were conducted in the absence of sodium, agonists and their corresponding antagonists have similar affinities for [^{3}H]naloxone binding sites in brain tissue. Interestingly, in guinea pig kidney membranes the agonists appear to be systematically more potent than the corresponding antagonists, which differ only by the substitution of *N*-allyl in the antagonist for *N*-methyl in the agonist (Table 5). For instance, in the guinea pig kidney morphine is about 20 times more potent than nalorphine, levorphanol is about 15 times more potent than levallorphan, and oxymorphone is about 3 times more potent than naloxone.

Detailed dose-response curves show some differences in the pattern of inhibition of [^{3}H]opiate binding by drugs in guinea pig kidney and brain (Figs. 2 and 3). Displacement curves of most agents in the brain are smooth and monophasic. In the guinea pig kidney inhibition of [^{3}H]naloxone and [^{3}H]dihydromorphine binding by levallorphan and dextrallorphan appears to be biphasic. Similar biphasic curves are apparent for inhibition of [^{3}H]naloxone binding by oxymorphone, naloxone, levorphanol, and levallorphan in guinea pig kidney, but not in brain. While [^{3}H]naloxone binding in brain is inhibited potently by 2-D-Ala-Met-enkephalin and β-endorphin, neither of these peptides affects guinea pig kidney binding of [^{3}H]naloxone over a wide range of concentrations.

Because of the anomalous properties of [^{3}H]opiate binding in guinea pig kidney, we evaluated numerous non-opiate drugs and endogenous substances. The butyrophenone-related agents haloperidol, benperidol, and pimozide have more affinity for kidney than brain binding sites. Pimozide, the most potent of these, displays an IC_{50} of 50 nM in guinea pig kidney. Since

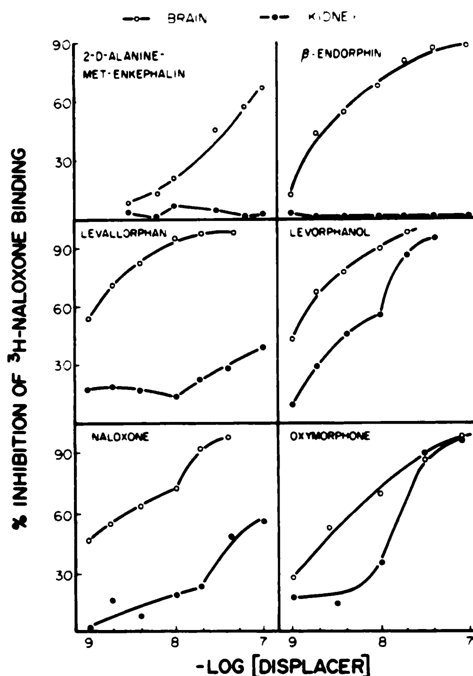

FIG. 3. *Reduction of [^{3}H]naloxone binding in guinea pig kidney membranes by opiate agonists, antagonists, and opioid peptides*

The standard assay contained 1.5 nM [^{3}H]naloxone.

drugs such as pimozide have high affinity for a number of receptor binding sites, it is difficult to evaluate the significance of these effects. Numerous endogenous peptides, amino acids, catecholamines, and several drugs known to be transported by specific renal mechanisms have no effect on [^{3}H]naloxone binding to guinea pig kidney membranes at a 5 μM concentration (Table 5).

The very low affinity of opioid peptides and of certain opiates for kidney binding sites raises the question whether these agents can bind to guinea pig kidney at all. Accordingly, we compared binding of [^{3}H]opiates and [^{3}H]enkephalin to guinea pig brain, liver, and kidney membranes (Table 6). All [^{3}H]ligands examined show substantial levels of specific saturable binding in brain membranes. Kidney and liver display considerable saturable binding for [^{3}H]dihydromorphine and [^{3}H]morphine. However, essentially no binding of [^{3}H]Met-enkephalin is apparent in kidney and liver. In addition, in other

TABLE 6

Effects of ions on binding of [^{3}H]opiates and [^{3}H]Met-enkepahlin to guinea pig tissues

The binding assay was performed at 25° with washed membrane preparations as described in MATERIALS AND METHODS. Data are from a typical experiment, which was replicated twice and whose results varied less than 20%.

[^{3}H]Ligand and ion added	Saturable binding		
	Brain	Kidney	Liver
	pmoles bound/g tissue		
[^{3}H]Naloxone 1.5 nM			
None	4.42	1.86	0.66
Na^+, 25 mM	5.14	1.84	0.60
K^+, 25 mM	3.99	1.48	0.70
Na^+, 100 mM	4.45	1.08	0.48
K^+, 100 mM	3.47	1.21	0.42
Mn^{++}, 1 mM	3.78	1.10	0.41
[^{3}H]Dihydromorphine, 1 nM			
None	2.08	1.68	0.46
Na^+, 25 mM	0.87	1.42	0.40
K^+, 25 mM	1.62	1.54	0.42
Na^+, 100 mM	0.65	1.02	0.16
K^+, 100 mM	1.58	0.88	0.32
Mn^{++}, 1 mM	2.37	1.15	0.36
[^{3}H]Morphine, 3.5 nM			
None	2.38	4.66	0.91
Na^+, 25 mM	1.48	3.35	0.65
K^+, 25 mM	2.35	3.84	0.65
Na^+, 100 mM	1.33	2.52	0.49
K^+, 100 mM	2.11	2.08	0.53
Mn^{++}, 1 mM	3.47	2.99	0.75
[^{3}H]Met-enkephalin 1.5 nM			
None	2.26	0	0
Na^+, 25 mM	1.21	0	0
K^+, 25 mM	2.69	0	0
Na^+, 100 mM	0.95	0	0
K^+, 100 mM	2.37	0	0
Mn^{++}, 1 mM	3.27	0	0

experiments, not depicted, no [^{3}H]-2-D-Ala-enkephalin binding is seen in kidney, indicating that enkephalin degradation is not responsible for the lack of binding.

Effects of ions on [^{3}H]opiate binding. One of the most dramatic features of the opiate receptor of nervous tissue is the selective influence of various ions (5–8). Under the present incubation conditions sodium has no effect on antagonist binding but selectively decreases agonist binding (6). Low concentrations of manganese enhance the binding of opiate agonists measured under appropriate conditions (7, 8). To compare further the characteristics of kidney and liver binding of opiates with brain receptors, we evaluated the influences of sodium, potassium, and manganese (Table 6). Sodium (25 or 100 mM) does not affect the binding of [^{3}H]naloxone but markedly reduces binding to brain membranes of [^{3}H]dihydromorphine and [^{3}H]morphine, while potassium has only a negligible effect. Unlike the selective influences of sodium in brain, in kidney and liver membranes 25 mM sodium and potassium have no significant effect on [^{3}H]dihydromorphine and [^{3}H]morphine binding. At 100 mM both sodium and potassium produce moderate decreases in [^{3}H]opiate binding and seem to affect the antagonist [^{3}H]naloxone as well as the agonists [^{3}H]dihydromorphine and [^{3}H]-morphine (Table 6).

Manganese (1 mM) has no influence on brain membrane binding of [^{3}H]naloxone, but enhances the binding to brain membranes of [^{3}H]dihydromorphine, [^{3}H]-morphine, and [^{3}H]Met-enkephalin, as reported previously (7, 8). By contrast, manganese decreases the binding of all [^{3}H]opiates to membranes of kidney and liver to a similar extent.

DISCUSSION

The major finding of this study is the presence of saturable binding of certain [^{3}H]opiates to membranes of guinea pig kidney and liver. The saturable binding in guinea pig small intestine presumably involves specific opiate receptors previously demonstrated to exist in this tissue (9). The specificity of this binding is indicated by its high affinity for certain agents and the fact that it cannot be demonstrated in several other tissues examined. Moreover, substantial levels of binding occur in guinea pig and rabbit membranes but not in certain other species.

Although the binding by guinea pig kidney membranes is affected by numerous opiates in a fashion somewhat similar to their influence on brain membrane binding, marked differences exist in drug specificity. Most pronounced of these discrepancies is the "reversed" stereospecificity of

guinea pig kidney [^{3}H]opiate binding for levallorphan and dextrallorphan. Also, agonists appear to be systematically more potent than antagonists in guinea pig kidney membrane binding. Interestingly, the opioid peptides examined have negligible affinity for guinea pig membranes, despite their great potency in the brain. Certain opiates, specifically etorphine and diprenorphine, behave like the peptides in lacking affinity for guinea pig kidney membranes. The binding sites in kidney and liver also differ from those of brain in failing to display characteristic responses to sodium and manganese.

Because of their anomalous properties, especially the reverse stereospecificity and the absence of selective sodium effects, it is unlikely that binding sites in kidney and liver mediate known major stereospecific pharmacological effects of opiates. To ascertain whether the binding sites might represent receptors for other endogenous compounds, we screened a variety of known peptides and neurotransmitters. However, we failed to detect any known naturally occurring substance with high affinity comparable to that of opiates for these binding sites. Because of the role of the kidney in transport processes, we speculated that the binding sites might represent transport binding sites. However, probenecid, ouabain, and a variety of transported drugs, including amino acids, various organic acids and bases (including uric acid), penicillins, salicylates, and sulfonamides, fail to inhibit binding at 5 μM concentration.

ACKNOWLEDGMENT

We thank Adele Snowman for outstanding technical assistance.

REFERENCES

1. Pasternak, G. W., Wilson, H. A. & Snyder, S. H. (1975) *Mol. Pharmacol.*, 11, 340–350.
2. Simantov, R. & Snyder, S. H. (1976) *Mol. Pharmacol.*, 12, 987–998.
3. Buscher, H. H., Hill, R. C., Romer, D., Cardinaux, E., Closse, A., Hauser, D. & Pless, J. (1976) *Nature*, 261, 423–425.
4. Snyder, S. H., Pasternak, G. W. & Pert, C. B. (1975) in *Handbook of Psychopharmacology* (Iversen, L. L., Iversen, S. D. & Snyder, S. H., eds.), Vol. 5, pp. 329–360, Plenum, New York.
5. Pert, C. B. & Snyder, S. H. (1974) *Mol. Pharmacol.*, 10, 868–879.
6. Simon, E. J., Hiller, J. M., Groth, J. & Edelman, I. (1975) *J. Pharmacol. Exp. Ther.*, 192, 531–537.
7. Pasternak, G. W., Snowman, A. M. & Snyder, S. H. (1975) *Mol. Pharmacol.*, 11, 735–744.
8. Simantov, R., Snowman, A. M. & Snyder, S. H. (1976) *Mol. Pharmacol.*, 12, 977–986.
9. Pert, C. B. & Snyder, S. H. (1973) *Science*, 179, 1011–1014.