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Physicochemical Basis of Opiate-Cerebroside Sulfate Interaction and Its Application to Receptor Theory

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SUMMARY

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Our postulate that opiate agonist and antagonist action is determined by the difference in the physicochemical properties between agonist-receptor complex and the complex formed with antagonist was tested using cerebroside sulfate as a model receptor. All of the drugs examined induced the [^{3}H]cerebroside sulfate transfer from an aqueous phase to a nonaqueous phase. However, most of the agonists were more effective in inducing the transfer than most relatively pure antagonists and in a series of homologues, the maximum transfer induced by the agonists was larger than that induced by the antagonists. Moreover, the transfer induced by levorphanol was reversed by various opiate antagonists, but not by agonists. Both the transfer induced by the drugs used and the inhibition by various types of opiate antagonists correlated closely with their analgetic potencies in man and their antagonisms of the agonist effects in the isolated guinea pig ileum, respectively. Partial agonists and antagonists exhibited biphasic effects; at the low concentration, they antagonized the agonist-induced [^{3}H]cerebroside sulfate transfer but at the higher concentration they induced their own transfer. When the [^{3}H]cerebroside sulfate transfer induced by agonists and the inhibition by antagonists were compared to that induced by inorganic cations, the tightness of the ionic interaction between the cationic nitrogen atom of the drug and the anionic sulfate group of CS could serve to explain the agonist efficacy, while hydration of the ionic bond determines the antagonist efficacy. Based on the molecular mechanism of opiate-cerebroside sulfate interaction, an opiate receptor theory was proposed.

INTRODUCTION

During the last decades, the influences of the basic nitrogen atom of the drugs on the agonist and antagonist activities have been greatly emphasized. The analgetic activity of various opiates decreases with increase in the effective width of the basic group (1) and the nitrogen atom plays a pivotal role in the mode of interactions with their receptor (2). The nature of the basic center is responsible for morphine-like and morphine antagonist effects (3, 4). The N-allyl group of the drug binds to either the agonist conformation of the receptor or the antagonist conformation depending on the conformation of the N-allyl group, whereas the N-phenethyl group of opiate interacts only with agonist conformation of the receptor (5, 6). All these postulates strongly suggest that the anionic site of the receptor is a prerequisite for agonist and antagonist effects. However, the precise molecular mechanism of the drug action via the inter-

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action between the basic center of the drugs and the anionic site of the receptor is not known yet.

Recently, we (7, 8) have proposed that based on the results of opiate binding to CS,[1] agonist and antagonist effects are elicited via the formation of hydrophobic intimate ion pairs and hydrophilic solvent separated ion pairs between the protonated nitrogen atom of the drug and the anionic site of the receptor, respectively. Although the opiate binding indirectly supports the proposed theory, binding *per se* does not discriminate between the two different ionic complexes. In this regard, we were looking for a method which could show directly the differences in the ionic properties between agonist-CS complex and the complex formed with antagonist.

Abramson *et al.* (9) have observed that the addition of univalent and divalent cations to an aqueous suspension of CS induces an increase in turbidity and the turbidity induced by Ca^{2+} is antagonized by monovalent cations (e.g., Li^+, Na^+, and K^+) at the lower concentrations which do not induce significantly the turbidity by themselves. Thus, depending on the concentration, the monovalent cations exhibited the biphasic effects by forming the two types of ionic complexes. Therefore, this method should be useful to test our proposed ionic receptor theory. However, the authors used 200 μg/ml CS. This concentration is too high to test the effect of opiate on CS turbidity since the concentration is well above the dissociation constants of opiate-CS complexes. Recently, we have found that the [^{3}H]CS transfer from the aqueous phase to the non-aqueous phase allows us to study the effects of opiate on the physicochemical properties of CS at very low concentrations of CS (10). The present paper describes our observations which can substantiate our proposed ionic receptor theory, using the method of [^{3}H]CS transfer.

MATERIALS AND METHODS

Chemicals. CS was purchased from Supelco Chemical Co. [^{3}H]CS (specific activity 3 Ci/mmole) was prepared by professor C. T. Peng (School of Pharmacy, Univ. of California, San Francisco) at the Lawrence Laboratories (Berkeley, CA), and purified by thin layer chromatography (11) using silica gel G (Anal Lab.) and solvent mixture (chloroform:methanol:water = 70:30:4). Drugs were donated by the following companies; Endo Laboratories, Garden City, N. Y. (naloxone, oxymorphone, nalbuphine, nalmexone, naltrexone, oxycodone); Hoffmann-La Roche, Nutley, N. J. (levorphanol, levallorphan, α-prodine); Sterling-Winthrop, Rensselaer, N. Y. (1-pentazocine, cyclazocine, meperidine); Ciba-Geigy, Summit, N. J. (GPA-1657, GPA-2163); American Cyanamid, Princeton, N. J. (diprenorphine, etorphine); Park Davis, Ann Arbor, Mich. (profadol); Bristol Lab., Div. of Bristol Myers Co., Syracuse, N. Y. (BC-2605) and Eli Lilly Lab., Indianapolis, Ind. (1-methadone). Ketobemidone, metazocine, etazocine, heroin, and normorphine were generous gifts from Drs. E. L. May and A. Jacobson, cyclorphan from Dr. H. W. Kosterlitz, and fentanyl and dextromoramide from Dr. P. Janssen. Nalorphine was purchased from Merk & Sharp Dohme, Rahway, N. J., morphine and codeine from Mallinckrodt.

Preparation of CS micelles. [^{3}H]CS was mixed with unlabelled CS in a mixture of chloroform and methanol (2:1) to give a specific activity of 281 mCi/mmole, and the solution was then taken down to complete dryness with a rotary vacuum evaporator on water bath at 50°C, after which the dried [^{3}H]CS was suspended in 10 ml of tris HCl buffer (100 mM, pH 7.4) and allowed to swell for 15 min. The [^{3}H]CS suspension was sonicated for 1 min and then diluted with the same buffer to give .4 μM.

[^{3}H]CS transfer studies. Five-tenths milliliter of the [^{3}H]CS micelles preparation was added to 0.5 ml of aqueous solution containing drug and/or cation at room temperature. This aqueous solution mixed with 0.3 ml of heptane on a vortex mixer (speed setting at 5) for 1 min and the mixture was allowed to stand for 20 min. A 0.5 ml aliquot of the aqueous phase mixed with 5 ml of Scintiverse cocktail solution (Fischer Scientific Co.) was assayed in a Beckman Liq-

[1] The abbreviation used: CS, cerebroside sulfate.

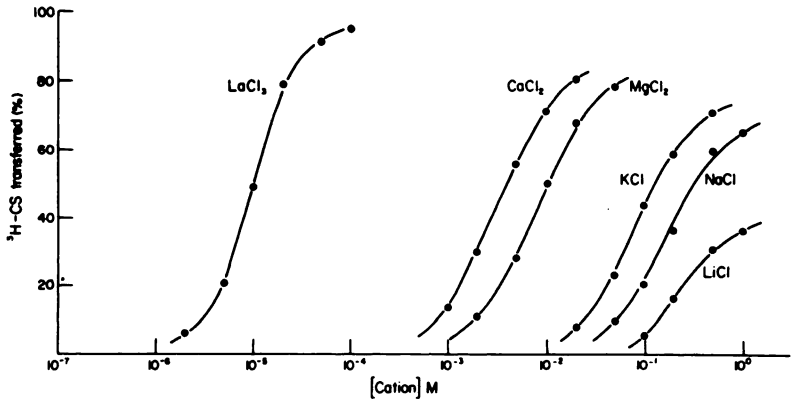


FIG. 1. *The effects of cations on [^{3}H]CS transfer*

Heptane-water partition method was used. System containing 0.3 ml of heptane and 1 ml of aqueous 2×10^{-7} M [^{3}H]CS (3 Ci/mmole) in 50 mM Tris·HCl (pH 7.4) with various concentrations of cations was shaken for 1 min at vortex speed 5 and allowed to stand for 10 min. An aliquot of aqueous phase (0.5 ml) was transferred to a counting vial containing 5 ml of cocktail solution and the radioactivity was counted by liquid scintillation spectrophotometry. The details of the experiment were described in METHODS. The radioactivity of the control in the aqueous phase was 14400 ± 200 cpm (40% of the total activity) and the maximum transfer induced by 100 μM of La^{3+} was 12240 ± 180 cpm (85% of the control value). The data were the mean of triplicate determinations.

uid Scintillation Counter to determine [^{3}H]CS radioactivity. The percent of drug-induced [^{3}H]CS transferred from the aqueous phase to the non-aqueous phase was calculated by using the following equation:

$$\%\ \text{of [}^3\text{H]CS transferred} = \frac{\text{radioactivity without drug in aqueous phase} - \text{radioactivity with drug or cation in aqueous phase}}{\text{radioactivity without drug in aqueous phase} - \text{radioactivity in the presence of 100 } \mu\text{M } La^{3+} \text{ in aqueous phase}^2}$$

RESULTS

The validity of this method was tested by comparing the concentration of cations to induce [^{3}H]CS transfer from the aqueous to non-aqueous phase with the data obtained by the method of turbidity (9). As shown in Fig. 1, the concentrations of cation required to induce [^{3}H]CS transfer fell in the order, $K^+ < Na^+ < Li^+$ which correlated well with the potencies of the cation-induced turbidity. Moreover, the Ca^{2+}-induced [^{3}H]CS transfer from aqueous phase to non-aqueous phase was antagonized by the addition of monovalent cations (Fig. 2). The potencies of the monovalent cations to antagonize the Ca^{2+}-induced [^{3}H]CS transfer were also parallel with those of the monovalent to reverse the turbidity of CS induced by Ca^{2+}. This indicates that the method of the [^{3}H]CS transfer in the heptane-water system is as good as that of turbidity in the aqueous system. Furthermore, the former is much more sensitive than the latter. Note that .2 μM of CS was used in this method instead of 200 μM CS used in the turbidity experiments. This concentration of CS is within the range of the apparent dissociation constants of various opiates (8) and so this method of [^{3}H]CS transfer could be used to test the effects of various opiates.

La^{3+} behaved like opiate agonists and the analgesia mediated by La^{3+} was antagonized by both Ca^{2+} and naloxone (12). It should be of interest, therefore, to see if the La^{3+}-induced [^{3}H]CS transfer is reversed by Ca^{2+} and naloxone. As shown in Fig. 3,

[2] La^{3+} at 100 μM induced the largest [^{3}H]CS transfer among the cations and drugs tested.

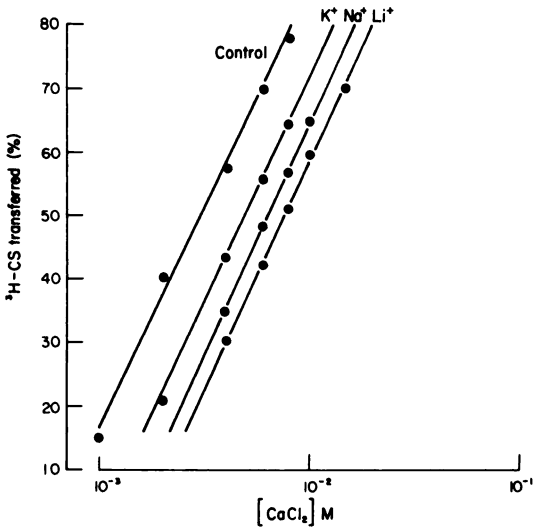


FIG. 2. *Antagonism of Ca^{2+} induced [^{3}H]CS transfer by monovalent inorganic cations*

Experiment was performed by the method described in Fig. 1. 50 mM of monovalent cations was used.

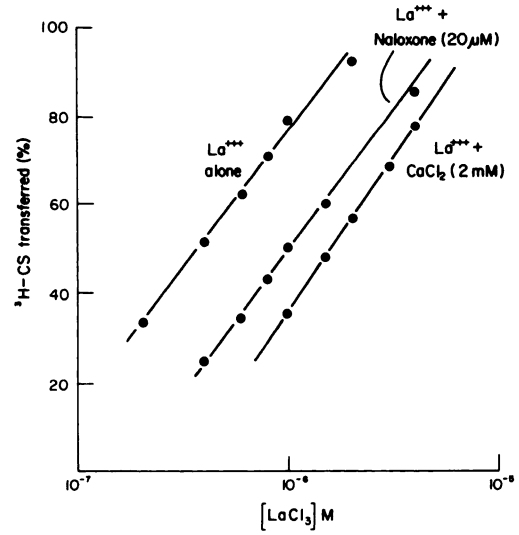


FIG. 3. *Antagonism of La^{3+}-induced [^{3}H]CS transfer by naloxone and calcium ions*

The experimental method is described in Fig. 1.

the effect of La^{3+} on [^{3}H]CS transfer was antagonized by both Ca^{2+} and naloxone at concentrations that did not significantly induce the transfer by themselves.

Since it has been proposed that the agonist and antagonist effects are determined by the resultant ionic complexes formed with the receptor (7, 8), it is relevant to observe the effects of opiate agonist and antagonist on [^{3}H]CS transfer. As shown in Fig. 4–Fig. 10, in a class of the compounds, each drug has its own characteristic concentration-response curves. The maximum transfers induced by agonists (e.g., etorphine, levorphanol, oxymorphone, morphine, metazocine and GPA-1657) were larger than those by their corresponding partial agonists (e.g., buprenorphine, cyclorphan, nalbuphine, nalorphine, cyclazocine, pentazocine, and GPA-3154) which in turn were larger than those by the corresponding pure antagonists (e.g., diprenorphine, naloxone and GPA-2163). The slopes of the curves for agonists were generally sharper than those of their corresponding antagonists. Thus, the behavior of agonist is similar to that of La^{3+}, while the antagonist behaves like Li^+ and Na^+ in inducing [^{3}H]CS transfer. To see if specificity and correlation between the opiate-induced [^{3}H]CS transfer and the analgetic activities of the drugs exist, the concentration of various compounds including opiate agonists and their antagonists, and neurotransmitters (over 50 compounds) required to induce the [^{3}H]CS transfer by 50% (EC_{50}) was determined. The data are shown in Tables 1–3. As shown in Fig. 11, the EC_{50}'s for agonists and partial agonists correlated well with their analgetic potencies in human ($r = 0.86$ for $n = 29$; $r = 0.92$ for $n = 27$ without codeine and meperidine). Neurotransmitters such as Ach., 5-HT, histamine and catecholamines are much less effective than opiate agonists and its partial agonists in inducing [^{3}H]CS transfer (Table 2).

The fact that the concentration–transfer curves of antagonists are similar to those of Li^+ and Na^+ suggests the possibility that the opiate antagonist can inhibit the [^{3}H]CS transfer induced by agonists. Fig. 12 shows that this is the case. For example, 20 μM of naloxone, which did not induce the [^{3}H]CS transfer on its own, antagonized the effects of levorphanol about 10 times. In order to determine the apparent dissociation constant (K_e) of the antagonists to reverse the effect of agonist, Kosterlitz's single dose method was used since this is

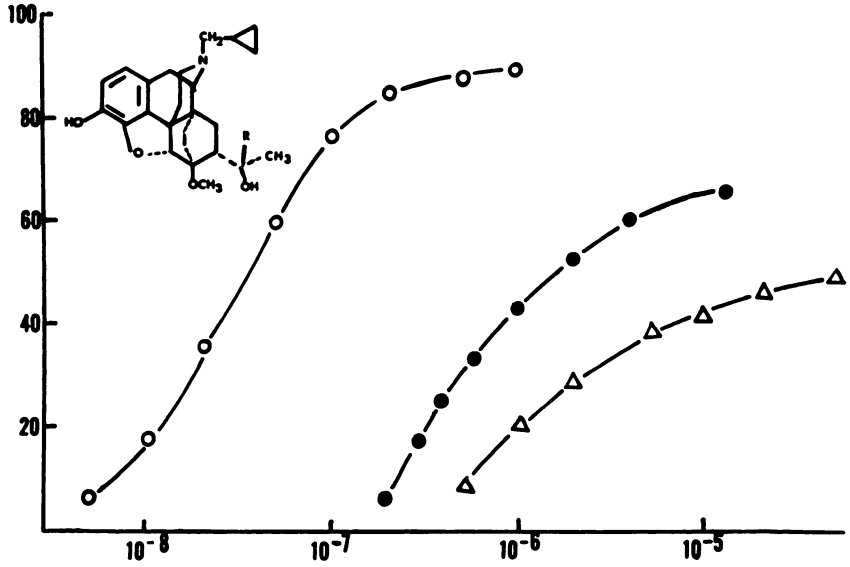


FIG. 4. *Concentration-[^{3}H]CS transfer curves of tetrahydrooripavine*

Ordinate: [^{3}H]CS, transferred (%). Abscissa: concentration of drug, M. Etorphine: R = $CH_2CH_2CH_3$, N-methyl instead of N-cyclopropylmethyl, and double bond between C_{15}-C_{16}. Agonist (-O-O-). Buprenorphine, R = —$C(CH_3)_3$: partial agonist (-●-●-). Diprenorphine, R = —CH_3: pure antagonist (-Δ-Δ-). The initial drug concentration in aqueous phase was used.

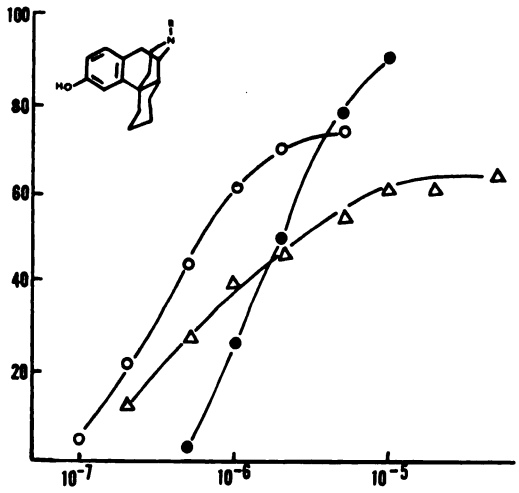


FIG. 5. *Concentration-[^{3}H]CS transfer curves of 3-hydroxymorphinan*

Ordinate: [^{3}H]CS, transferred (%). Abscissa: concentration of drug, M. Levorphanol, R = —CH_3: agonist (-●-●-). Cyclorphan, R = -CH_2-<: partial agonist (-O-O-). Levallorphan, R = —$CH_2CH{=}CH_2$: partial agonist (-Δ-Δ-Δ-).

reported to be a good method to determine antagonist activities for partial agonists in the isolated guinea pig ileum (14, 15). As shown in Table 3 and Fig. 13, the K_e values of antagonists were much lower than their EC_{50} and correlated with their *in vitro* antagonist activities observed in the isolated guinea pig ileum as well as the *in vivo* activities (16). The ratio of the EC_{50} for partial agonists to the K_e values was parallel with the efficacy of antagonist in the isolated guinea pig ileum (Table 3). To see if agonists could also antagonize the levorphanol-induced transfer, morphine, oxymorphone, and methadone were used at the concentration (10^{-6} M) which did not significantly induce their own transfer. None of the agonists examined reversed the effect of levorphanol. Figure 14 shows that the [^{3}H]CS transfer induced by opiate agonist is antagonized by sodium ion as in the case of opiate antagonist.

DISCUSSION

Molecular mechanism of the drug-CS interaction. Both the relative EC_{50}'s of opiate agonists including partial agonists (Table 1 and Fig. 11) and relative K_e values of the antagonists (Table 3 and Fig. 13) correlated closely with their analgetic poten-

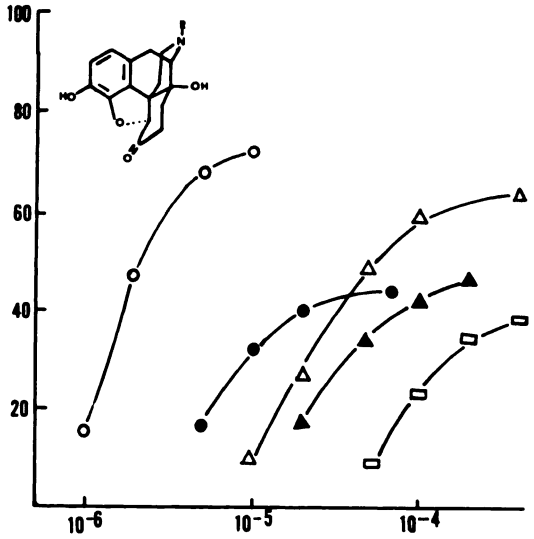


FIG. 6. *Concentration-[^{3}H]CS transfer curves of N-substituted noroxymorphone*

Ordinate: [^{3}H]CS, transferred (%). Abscissa: concentration of drug, M. Oxymorphone, R = —CH_3: agonist (-O-O-). Naltrexone, R = -CH_2-◁: relatively pure antagonist (-●-●-). Nalbuphine, R = -CH_2-◇ partial agonist (-△-△-). Nalmexone, R = —CH_2-CH=C(CH_3)$_2$: partial agonist (-▲-▲-). Naloxone, R = —CH_2—CH=CH_2: pure antagonist (-□-□-).

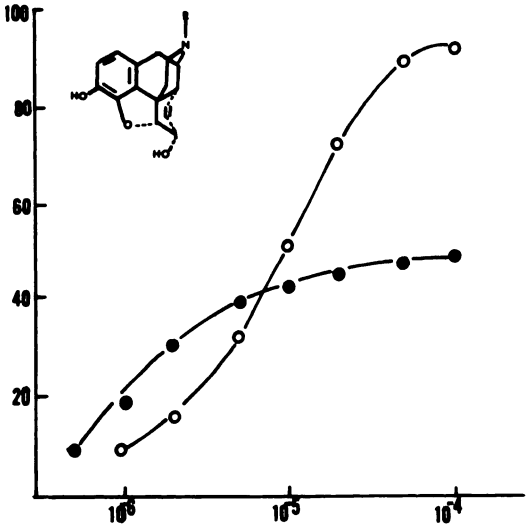


FIG. 7. *Concentration-[^{3}H]CS transfer curves of N-substituted normorphine*

Ordinate: [^{3}H]CS, transferred (%). Abscissa: concentration of drug, M. Morphine, R = —CH_3: agonist (-O-O-). Nalorphine, R = —CH_2—CH=CH_2: partial agonist (-●-●-).

cies and inhibition of the effects, respectively. Moreover, the ratio of EC_{50} to K_e values was parallel with the antagonist efficacy. These suggest that the molecular mechanisms of the opiate-CS interaction may be similar to that of the opiate-receptor interaction. Therefore, the mechanism of the [^{3}H]CS transfer induced by agonists and its inhibition by antagonists should be a useful model to obtain insight into the molecular mechanism of opiate agonist and antagonist actions.

It seems clear that there are two types of drug (or cation)-CS complexes: hydrophobic and hydrophilic complexes. The hydrophobic complexes were evident from the induction [^{3}H]CS transfer while the hydrophilic complexes were manifested from the antagonism of [^{3}H]CS transfer by the monovalent cations and antagonists. The fact that at the low concentrations below those required to transfer CS, partial agonists and antagonists inhibit the CS transfer induced by agonist indicates that these drugs predominantly form hydrophilic complexes with CS at the low concentrations. However, at the higher concentrations required to induce the transfer, both hydrophilic and hydrophobic complexes are in equilibrium and the ratio of the hydrophobic agonist-CS complex to the hydrophilic one is also

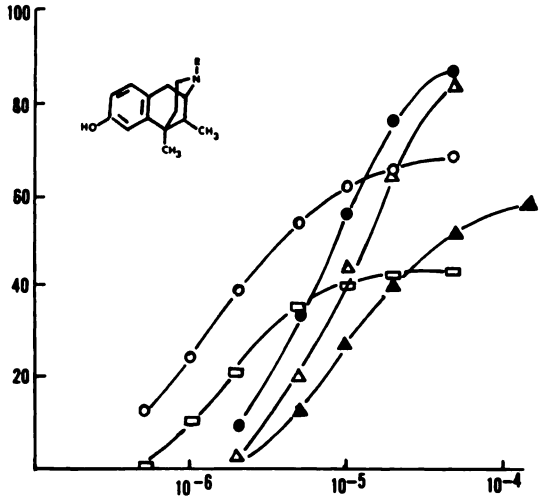


FIG. 8. *Concentration-[^{3}H]CS transfer curves of 2R,5,9-dimethyl-2'-hydroxy-6,7-benzomorphan*

Ordinate: [^{3}H]CS, transferred (%). Abscissa: concentration of drug, M. Metazocine, R = —CH_3: agonist (-●-●-). Etazocine, R = —CH_3, 5,9-diethyl instead of dimethyl: agonist (-△-△-). Cyclazocine, R=-CH_2-◁: partial agonist (-○-○-). Pentazocine, R = —CH_2—CH=$C(CH_3)_2$: partial agonist (-▲-▲-). N-Allylnormetazocine, R = —$CH_2CH{=}CH_2$: relatively pure antagonist (-□-□-).

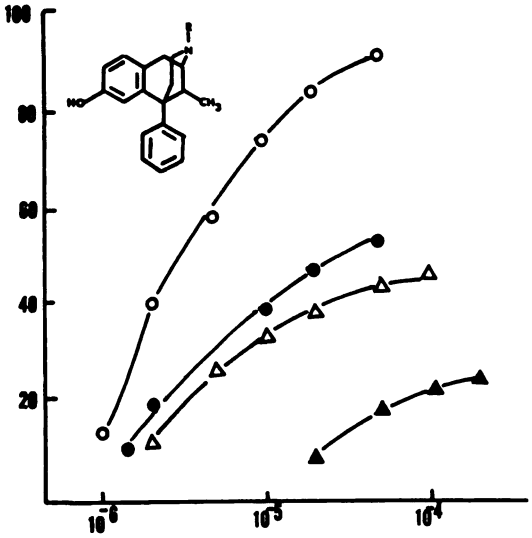


FIG. 9. *Concentration-[^{3}H]CS transfer curves of 2R, 5-phenyl-9-methyl-2'-hydroxy-6,7-benzomorphan*

Ordinate: [^{3}H]CS, transferred (%). Abscissa: concentration of drug, M. GPA-1657, R = —CH_3: agonist (-○-○-). GPA-3154, R=-CH_2-◇: partial agonist (-●-●-). GPA-2443, R = —CH_2—CH=$C(CH_3)_2$: partial agonist (-△-△-). GPA-2163, R = —CH_2—C≡CH: pure antagonist (-▲-▲-).

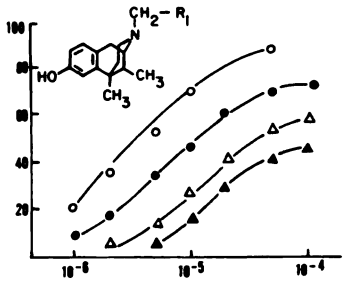


FIG. 10. *Concentration-[^{3}H]CS transfer curves of 2R,5,9-dimethyl-2'-hydroxyl-6,7-benzomorphan*

Ordinate: [^{3}H]CS, transferred (%). Abscissa: concentration of drug, M. Mr-1353, R = 2'-(3'-methyl)furyl: agonist (-○-○-). Mr-1268, R = 3'-(2'-methyl)furyl: partial agonist (-●-●-). Mr-1029, R = 2'-furyl: partial antagonist (-△-△-). Mr-1256, R = 3'-furyl; relatively pure antagonist (-▲-▲-).

higher than that of the partial agonists or that of antagonists in a homologue series, since the maximum transfer of agonist is generally higher than that of the partial agonist or that of antagonist (Figs. 4–10).

Based on the above discussion, the general molecular mechanism of the drug-CS interaction could be summarized as follows:

$$D \overset{P}{\rightleftharpoons} D_a^+ + CS^- \overset{K_D}{\rightleftharpoons} \text{DCS (hydrophilic)} \overset{k}{\rightleftharpoons} \text{DCS* hydrophobic)}$$

The drug (D) in bulk solution is adsorbed on the interface between heptane (or

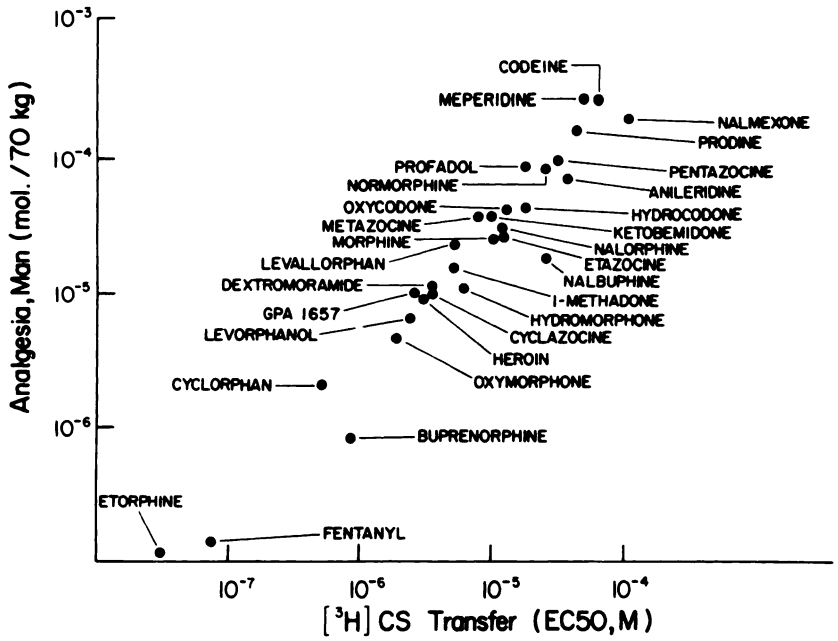


FIG. 11. *Correlation between the agonist potencies of narcotic analgesics in man and in [^{3}H]CS transfer*
The correlation was determined by the method of linear regression. The correlation coefficient (r) = 0.86 for n = 29; r = 0.92 for n = 27 without codeine and meperidine.

aqueous micelles) and water and the adsorbed form (D_a^+) interacts with CS. $P = D_a^+/D'$ K_D = dissociation constant and k = DCS*/DCS.

The molecular mechanisms for pure agonists and antagonists simplify as follows:

$$D \rightleftharpoons D_a^+ + CS^- \rightleftharpoons DCS^* \quad \text{Agonist } (k \gg 1)$$

$$D \rightleftharpoons D_a^+ + CS^- \rightleftharpoons DCS \quad \text{Antagonist } (k = 0)$$

However, it should be mentioned that the pure agonists and antagonists in this system were not found as evident from the maximum transfer.

Relationship between efficacy and the ionic nature of the complex. In a homologous series (Figs. 5–9), the substitution of N-methyl group by allyl, cyclopropylmethyl or dimethylallyl groups decreased the maximum transfer. One would expect, according to the Hansch analysis, that the maximum transfer would be increased by such substitution, since the lipophilicity should be increased. Contrasted to this, the maximum transfer was attenuated by the substitution. The substituted groups are larger in size and more polarizable than the methyl group. Polarizability could contribute to hydration. For example, it is well known that unsaturated gas anesthetic such as ethylene and propylene are water crystal forming agents (17, 18). Both increase in size, and hydration increases the effective size of the cation, resulting in the decrease of the ion pair formation between the positive charge of the drug and the sulfate group of CS. On the other hand, hydration of the allyl group could stabilize the hydrophilic complex that can antagonize the [^{3}H]CS transfer induced by agonists.

A similar conclusion can be obtained from the differences in pharmacological properties between the N-methyl substituents of opiates and the N-allyl derivatives. For example, in the pairs of morphine and nalorphine, the two drugs had similar analgetic potencies (Table 1) when administered intravenously but a smaller dose (2–5 mg) of nalorphine antagonized the effects of morphine (19). Taking into account that the nalorphine concentration in the brain is

TABLE 1

Comparison of analgetic potencies of narcotic analgesics with their EC_{50}'s

	Analgesia (man)[a]		[^{3}H]CS Transfer	
	μmol/70 kg	r[k]	EC_{50} (μM)	r[k]
Etorphine	0.11[b]	239.6	0.04	250
Buprenorphine	0.79[c]	33.4	0.85	11.8
Fentanyl	0.13[b]	202.8	0.07	142
Dextromoramide	11.66[d]	2.3	3.5	2.9
l-Methadone	14.45	1.8	5	2.0
β-Endorphin	—	—	0.15	66.7
Oxymorphone	4.33	0.13	1.8	5.6
Nalmexone	199.56[e]	0.13	100	0.10
Nalbuphine	19.96[f]	1.3	25	0.4
Hydromorphone	10.88	2.4	6	1.7
Oxycodone	41.57	0.63	12	0.83
Hydrocodone	44.67	0.59	17	0.59
Meperidine	264.26	0.10	45	0.22
α-Prodine	167.89[b]	0.16	42	0.24
Anileridine	70.51[b]	0.37	35	0.29
Ketobemidone	35.24	0.75	9.6	1.0
Profadol	87.86[g]	0.30	19	0.53
BC-2605	—	—	15	0.67
Morphine	26.36	1.0	10	1.0
Nalorphine	28 75	0.92	12	0.83
Normorphine	81.20[h]	0.32	25	0.40
Heroin	9.86	2.7	3	3.3
Codeine	267.98	0.1	60	0.17
Levorphanol	6.12	4.3	2.4	4.2
Levallorphan	23.03[b]	1.1	5	2.0
Cyclorphan	2.03[i]	13.0	0.5	20.0
GPA-1657	10.10[g]	2.6	2.5	4.0
GPA-3154	—	—	13	0.77
GPA-2443	—	—	40	0.25
Metazocine	37.34[b]	0.70	8	1.3
Etazocine	27.04[b]	0.97	12	0.83
Cyclazocine	9.75[j]	2.7	3.5	2.9
Pentazocine	93.2 [b]	0.28	30	0.33
Mr-1353	—	—	5	2
Mr-1268	—	—	15	0.67
Mr-1029	—	—	40	0.25
Mr-1256	—	—	70	0.14

The data were taken from the following references and converted mg into μmole.

[a] (24), unless otherwise indicated; [b] (13); [c] (25); [d] (26); [e] (27); [f] (28); [g] (29); [h] (30); [i] (31); [j] (32); [k] r = relative potency (morphine = 1).

TABLE 2

EC_{50}'s of neurotransmitters

Neurotransmitters	EC_{50} (μM)
Norepinephrine	160
5-Hydroxytryptamine	100
Dopamine	160
Histamine	340
Acetylcholine	4500

10 times higher than that of morphine after the equal dose of i.v. injection (4), the interaction of morphine with the receptor is considerably stronger than that of nalorphine to the receptor. Since the only structural difference between the two drugs is at the nitrogen substitution, one can presume that the N-allyl group of nalorphine produces more steric interference with the in-

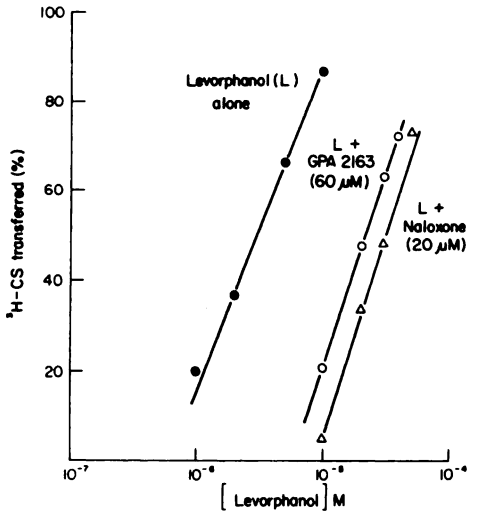


FIG. 12. *Antagonism of levorphanol-induced [³H]CS transfer by opiate antagonists*

The experimental methods are same as the method described in Fig. 1.

TABLE 3

EC_{50} and apparent dissociation constants (K_e) of relatively pure opiate antagonists and partial agonists

Drugs	EC_{50} (μM)	K_e (μM)	EC_{50}/K_e	
Diprenorphine	20	0.18	111	R
Naltrexone	>40	0.54	>74	R
BC-2605	15	0.72	21	P
Levallorphan	5	0.78	6.4	P
Naloxone	>150	1.4	>107	R
Cyclazocine	3.5	1.0	3.5	P
Nalbuphine	25	3.8	6.6	P
Nalorphine	12	2.9	4.1	P
GPA-2163	>200	10.1	>20	R
Nalmexone	100	10.05	10	P

R = relatively pure antagonist.
P = partial agonist.

timate ion pair formation between the positive charge of the drug and the negative charge of the receptor than the N-methyl group of morphine; but at the lower dose (2–5 mg), nalorphine, for the same reasons mentioned above, would form a solvent separate ion pair which can antagonize the analgetic effect of morphine. Several investigators have similarly suggested that the nature of basic center is responsible for the difference in pharmacological effects between agonists and antagonists (3, 4, 20).

The involvement of N-substituent hydration in the maximum transfer is further supported by N-furylmethyl analogues in Fig. 10, since hydration of the oxygen lone pair electron in the furan ring of benzomorphins could account for the difference in maximum transfer. The fact that MR-1029 is more lipophilic than MR-1256 (21) sug-

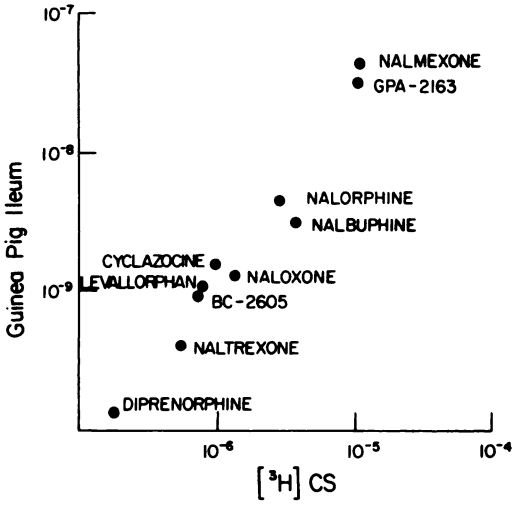


FIG. 13. *Relationship between the dissociation constants (K_e) of narcotic antagonists in the guinea pig ileum (16) and in the inhibition of [³H]CS transfer (Table 3)*

Correlation coefficient = 0.96 for n = 10.

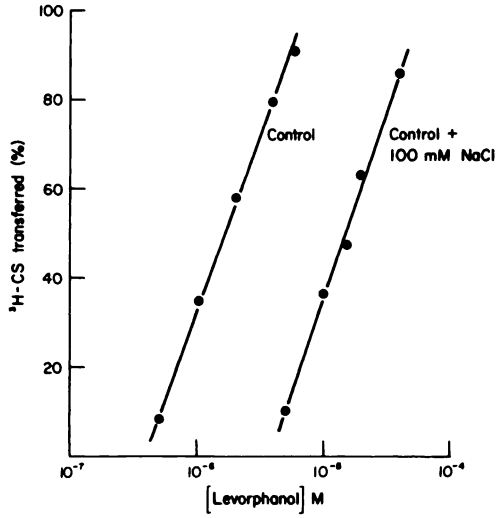
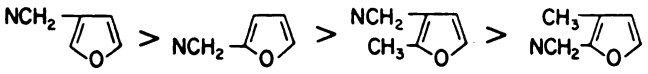


FIG. 14. *Antagonism of levorphanol-induced [³H]CS transfer by sodium ion*

gests that both N-methylene and methyl groups on the furan ring sterically hinder hydration of the lone pairs; the steric effects of the larger N-methylene group should be greater than the methyl group and the same alkyl group at the 2-position is more effective than that at the 3-position. Therefore, one could predict that the order of hydration is:



MR-1256 MR-1029 MR-1268 MR-1353

This order is inversely proportional to the maximum transfer (Fig. 10) and the agonist efficacy in analgetic activity but proportional to antagonist efficacy (33).

In the case of the oripavine series (Fig. 4), the involvement of the ionic bond in maximum transfer seems difficult to explain since the maximum transfer is changed by stereochemistry and alkyl substitution at the 19-carbon atom which is remote from the nitrogen atom. However, there is supportive evidence. Recently, Loew *et al.* (20) have suggested, based on the side chain conformation calculated by the quantum chemical method, that the 19-R propyl group of oripavine binds to a lipophilic receptor site and this interaction imposes a change in N-substituent binding. The change could interfere with antagonist type of N-substituent binding mode while enhancing the agonist type (20).

The effects of inorganic cations on [^{3}H]CS transfer also support our interpretation. The potency of the inorganic cations required to induce the [^{3}H]CS transfer (Fig. 1) was proportional to the charge density of the cation and inversely proportional to the hydrated size of the cations (22). Moreover, the order of the cationic selectivity in inducing the transfer is the same as that of affinity for both organic and inorganic sulfates (23). These indicate that the hydrophobicity of the cation-CS complexes (salt) are determined by the strength of the ionic interaction between the cation and the anionic oxygen atoms of the sulfate in CS. However, the fact that the effectiveness of the monovalent cations to reverse the Ca^{2+}-induced [^{3}H]CS transfer are parallel with the hydrated size of the cations (Fig. 2) suggests that the hydrophilicity of the complex is not directly associated with the strength of the ionic bond but with the degree of hydration.

All these interpretations seem to support our idea that the efficacy is determined by the ionic bond between the opposite charges. However, such substitution, in addition to the ratio (k), could influence the availability of a drug (P), resulting in the change in potency. The apparent effects of the substituents on the availability (P) can be seen from the fact that at low concentrations, partial agonists often gave more transfer than agonists (Figs. 5, 7, 8). This could be due to the large increase in the availability (P) compared to the ratio (k) since the difference in the concentration-transfer curves in Figs. 5, 7 and 8 can be generated using the suggested molecular mechanism.

In conclusion, the opiate-CS interactions correlated well with their pharmacological effects and CS discriminated between agonists and antagonists. The molecular mechanism of opiate-CS interaction is useful to obtain further insight into the mechanism of opiate action. However, whether or not CS is a component of opiate receptor remains to be determined.

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