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COMMENTARY

NEUROCHEMICAL BASES FOR NARCOTIC TOLERANCE AND DEPENDENCE*

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Recent efforts in attempts to elucidate the neurochemical mechanisms of narcotic actions have centered on the isolation and characterization of narcotic (opiate) receptors and the study of compounds which alter narcotic abstinence syndrome, tolerance and/or physical dependence [1]. There have been several recent short reviews on opiate receptors which have been written by the principals who are actively involved in this research [2–4]. A monograph [5] on opiate receptor mechanisms is also available which was based on a Neurosciences Research Program Work Session held in 1974. Another monograph on a similar topic based on a conference of the International Narcotic Research Club (INRC) will be forthcoming in *Life Sciences* [6].

NARCOTIC (OPIATE) RECEPTORS

Narcotic analgesics are generally believed to exert their pharmacologic effects by interacting with specific receptors located in the central nervous system. The presence of specific narcotic receptors is generally assumed because narcotic drugs have common structural features, enantiomers of narcotic drugs exhibit large potency differences, and structurally related, specific narcotic antagonists are available.

Taking advantage of the fact that most synthetic narcotic analgesics are the *l*-isomer, while their *d*-isomers are either inactive or very much less active, Goldstein *et al.* [4] demonstrated in 1971 the stereospecific binding of levorphanol to brain fractions and speculated that the binding material may be opiate receptors. The stereospecific binding represented only 2 per cent of the total binding. They further employed Sephadex LH-20 columns to partially purify the binding material which was completely extractable in chloroform–methanol and had the general properties of a proteolipid. After radioactive levorphanol was reacted with the binding material and the complex was fractionated on Sephadex LH-20 columns, the peak of radioactivity emerged at a more lipophilic region. Binding experiments indicated that this material exhibited at least three distinct stereospecific binding capacities with different binding affinities suggesting the presence of several kinds of stereospecific opiate receptors.

The availability of radiolabeled narcotics and antagonists with high specific activity has made possible the demonstration independently by three groups of investigators [2, 3, 7] in 1973 of the stereospecific binding of these compounds to brain homogenates or fractions with very low dissociation constants. Stereospecific binding in these instances now became a major portion of the total binding. These authors also believed that the binding represented the demonstration of the opiate receptor. Snyder *et al.* [3] used as their radioactive ligand the narcotic antagonist, ^{3}H-naloxone, while Simon's group [2] used the very potent narcotic agonist, ^{3}H-etorphine. Levorphanol markedly inhibited the binding of the ligands, whereas the inactive *d*-isomer, dextrorphan, did not. Terenius [7] employed [^{3}H]dihydromorphine as the ligand and determined stereospecific binding by the use of *l*- and *d*-methadone.

Snyder *et al.* [3] studied the affinities of a large number of drugs for the naloxone binding sites by the determination of the concentration of the drug required to decrease the specific binding of 5×10^{-9} M [^{3}H]naloxone by 50 per cent (ED_{50}). The binding affinities of various narcotic analgesic and antagonists generally paralleled their pharmacologic potencies. It was established that none of a wide variety of non-opiate drugs had any affinity for the naloxone binding site. The ED_{50} values estimated by competition of drugs for specific binding of 3×10^{-9} M [^{3}H]etorphine by Simon's group [2] generally agreed with those of Snyder *et al.*

One of the differences Simon [5] noted was the marked inhibition of etorphine binding by increasing salt concentration, whereas salt concentration had no effect on the naloxone binding found by Snyder *et al.* Simon suggested that this salt effect may denote the different manner in which narcotic analgesics and antagonists bind to receptors. Snyder's group [5] showed that the suggestion was generally true. In the presence of increasing sodium ions, the binding of antagonists was increased, while that of agonists decreased. From analysis of binding kinetics of agonists and antagonist, Snyder's group reported that sodium increased the number of antagonist binding sites and decreased the number of agonist binding sites. Scatchard plots also indicated that agonists and antagonists were bound by a high and a low affinity binding component differing in affinity by about 100, and the effects of sodium were exerted primarily on the number of high affinity sites. On the other hand, the data of Simon [5] indicated that the enhancement of antagonist binding due to sodium was the result of an increased affinity constant rather than an increase in binding sites. Both groups agree that the

* Literature surveyed to May 1975.

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receptors exist in two interconvertible conformational forms, a "sodium" and a "no sodium" conformation. Pert and Snyder [5] offered the possibility of using the sodium effect as a predictor of relative agonist–antagonist properties of drugs *in vivo*.

The regional distribution of stereospecific binding of radiolabeled etorphine in human brains and radiolabeled dihydromorphine in monkey brains has been studied [5]. Over 40 anatomical regions were examined and the areas with the highest binding capacity were those associated with the limbic system in both human and monkey brains.

With regard to the role of the stereospecific binding material in the phenomena of narcotic tolerance and dependence, Terenius [7] did not find any change in the stereospecific binding when animals were treated with morphine for 4 days. Using brain preparations from animals which were made highly tolerant and dependent by subcutaneous implantation of morphine pellets, neither Klee and Streaty [8] nor Hitzmann *et al.* [9] were able to demonstrate consistently any changes in binding affinity or capacity which correlated with the development of tolerance or physical dependence. Pert *et al.* [10] found that administration of either narcotic agonists or antagonists to mice enhanced the stereospecific binding capacity of brain extracts within several min after the injection. Since the same type of effect was seen in mice implanted with morphine pellets for various times, they concluded that the enhanced receptor binding was unrelated to tolerance and physical dependence.

A most notable finding by Loh *et al.* [11] is that narcotic agonists and antagonists could be bound stereospecifically to commercially available cerebroside sulfate. The affinities of various narcotic analgesics paralleled the pharmacologic potencies of these compounds in mice as well as man in a manner similar to that seen with the stereospecific binding material from brain [6].

Loh [6] compared the properties of commercial cerebrosides to the partially purified "opiate receptor" described by Goldstein [4]. Fractionation on Sephadex LH-20 columns revealed that cerebroside sulfate emerged from the column in the same exact location as Goldstein's "receptor." When the cerebroside sulfate–levorphanol complex was fractionated, it migrated to a more lipophilic area just like the "receptor"–levorphanol complex. Loh further showed that most of the apparent amino nitrogen of the purified "receptor" could be accounted for by the nitrogen content of cerebroside sulfate. He also demonstrated by careful analysis that the "receptor" fraction contained no proteins or amino acids. Although cerebroside sulfate has the highest stereospecific binding capacity, Loh's group has demonstrated that levorphanol binds stereospecifically to other acidic lipids such as phosphatidic acid, phosphatidylinositol, triphosphoinositide, phosphatidylserine and ganglioside (personal communication).

While it is true that the binding functions of various narcotic analgesics to the binding material in brain are generally correlated with pharmacological potencies of the drugs (ED_{50}), it has been pointed out [1] that such a correlation may not be strictly valid. Analgesic potency of a drug that is administered parenterally is influenced by several processes which determine the bioavailability of the drug at the receptor site in the central nervous system. Thus, a comparison of the binding to brain concentrations of the narcotics after an equieffective dose would be more meaningful. A potency ranking of various narcotic analgesics according to their brain concentrations has been made by Herz and Teschemacher [12] and this type of ranking becomes very different from the ranking of the analgesic ED_{50} values. When binding functions of narcotics are compared to analgesic potencies on the basis of brain concentration, the correlation is substantially weakened. Terenius [13] has indicated that binding affinities of narcotics correlate much better with analgesic potencies after intracerebroventricular injections than after intravenous injections.

To further insure the pharmacological relevance of the stereospecific binding material, Snyder's group [5] compared the binding "ED_{50}" and analgesic ED_{50} value of a homologous series of *N*-alkylnorketobemidones. The side chain length varied from methyl to decyl which produced marked variations in analgesic potencies. Similar variations in binding affinities were observed, with the greatest binding as well as the analgesic activity occurring in the pentyl derivative. Even more remarkable is the fact that Loh's group [6] has been able to demonstrate that the binding of the same homologous series of compounds to cerebroside sulfate showed the same variations in binding affinities with the greatest binding seen with the pentyl derivative. With such similarities between the stereospecific binding material of brain and cerebroside sulfate, stimulus should be provided to delineate the possible role cerebroside sulfate plays in opiate receptor mechanisms. Loh has proposed cerebroside sulfate as a useful model for studying molecular mechanisms involved in narcotic action.

POSSIBLE "ENDOGENOUS LIGAND" FOR NARCOTIC RECEPTORS

Since morphine is a plant product, many investigators have asked the question, why postulate opiate receptors and, if present in the body, what is their physiological role? The most intriguing advance in answering these questions is the discovery of a possible "endogenous ligand" in the brain for the opiate receptors which was first reported by Terenius and Wahlström [14] and Hughes [15] and communicated at the Neurosciences Work Session [5] and the symposium of the INRC [6] by Hughes, Terenius, and Pasternak and Snyder. The water-soluble, heat-stable "endogenous ligand" competes for [^{3}H]dihydromorphine sites on the stereospecific binding material (Terenius) or for [^{3}H]naloxone binding sites (Pasternak and Snyder). The "endogenous ligand" appears to act like a narcotic agonist both in the bioassay with the mouse vas deferens (Hughes) and in the binding assay in the presence of sodium (Pasternak and Snyder). Hughes and Pasternak *et al.* [6] showed that the ligand has a regional distribution in the brain which is similar to that for opiate binding material. The molecular weight of the "endogenous ligand" has been estimated to be about 1000 by all three groups of investigators. The ligand is rapidly destroyed by treatment with carboxypeptidase-A and leucine

aminopeptidase, but is relatively stable when treated with trypsin or chymotrypsin. The "endogenous ligand" has been coined NRA (naloxone reversible activity) or enkephaline by Hughes and MLF (morphine-like factor) by Terenius and Pasternak and Snyder.

The discovery of another peptide-like substance from the pituitary that acts like morphine was first communicated from Goldstein's laboratory at the INRC symposium [6]. This material acts like morphine in bioassays with either the stimulated guinea pig ileum or mouse vas deferens and inhibits etorphine or naloxone binding to putative opiate receptors. This material has been called POP_1 (pituitary opiate peptide 1). POP_1 appeared to be different from enkephaline or MLF in that both trypsin and chymotrypsin destroyed its activity. The molecular weight of POP_1 has also been estimated to be between 1500 and 2000 by gel filtration. The purification and characterization of these "endogenous ligands" should open up many new avenues for future research on narcotic receptors. In light of these findings, analgesia and morphine-like tolerance produced by focal electrical stimulation [16] may take on added significance.

PHARMACOLOGIC CHARACTERIZATION OF NARCOTIC RECEPTORS

Our group has in recent years applied the concept of pAx in intact animals for the characterization of analgesic receptors [1, 17]. The definition of the apparent pA_2 *in vivo* then becomes the $-\log$ of the molar dose of the injected antagonist which reduces the effect of a double dose of an agonist to that of a single dose. Although pA_2 is theoretically equal to the log of the affinity constant (K_B) of the antagonist for the receptor, this is not entirely true *in vivo*. However, the K_B *in vivo* should be proportional to the real K_B if it is assumed that the concentration of the antagonist at the receptor site is proportional to the injected dose, which is a likely assumption from pharmacological data. The consistently reproducible apparent pA_2 value for morphine–naloxone in different experimenter's hands, laboratories, strains of mice and analgesic assay procedures attests to the utility of the apparent pA_2 value as a pharmacologic constant.

Using the relatively pure antagonist naloxone, the apparent pA_2 values for a group of narcotic-type and narcotic-analgesic-type analgesics were determined in mice. The narcotic agents, morphine, methadone and levorphanol, displayed similar apparent pA_2 values, indicating that these agents probably interacted at similar receptors. The narcotic-antagonist analgesics, pentazocine, nalorphine and cyclazocine, also exhibited similar pA_2 values. However, the pA_2 values for the narcotic-antagonist analgesics were significantly lower than those for the narcotic agents. It was concluded that the two types of analgesics interacted either with two different receptor populations or with the same receptor in a different manner.

The significant increase of pA_2 values of morphine–naloxone in morphine-pretreated mice suggested that morphine induces some type of qualitative change in analgesic receptors. Mice were pretreated with a $> ED_{99}$ dose of morphine, and after 2 hr when the analgesic effect of morphine was no longer evident, dose–response curves for morphine in the absence and presence of three increasing doses of naloxone were determined using both the writhing and tail-flick analgesic assays. Naloxone shifted the dose–response curves substantially more to the right than when control mice were used, i.e. the animals had become much more sensitive to naloxone. The apparent pA_2 value increased significantly from 6·96 to 7·30 which represented over a doubling of the affinity constant. The increased sensitivity to naloxone was induced by all narcotic agonists but not by the inactive isomer of levorphanol, dextrorphan, a narcotic-antagonist analgesic like pentazocine, or naloxone itself. The above data again suggest that the interaction of narcotic-antagonist analgesics and narcotic agents with the receptor is not the same. The increased sensitivity to antagonists can also be observed when one uses nalorphine or diprenorphine as antagonists instead of naloxone.

These findings suggested the possibility of using this measure as a sensitive indicator of the initiation and development of tolerance. Observations in animals which were gradually made morphine-tolerant by injections and morphine-pellet implantation revealed that increased sensitivity to naxolone is observed earlier than analgesic tolerance, and over the course of the development of tolerance, the change in potency of antagonists rises much faster than the development of tolerance. When the animals become highly tolerant, the development of tolerance parallels the enhanced potency of naloxone. Upon abrupt withdrawal of the morphine administration, the tolerance and enhanced naloxone sensitivity rapidly fall together. That the observed naloxone sensitivity in tolerant animals is also an index of the development of physical dependence is indicated by the fact that an inverse relationship between the degree of dependence and the ED_{50} of naloxone to precipitate withdrawal jumping in mice has been shown by Way's group [18, 19]. Fishman *et al.* [20] have shown, using radioactive compounds with very high specific activity, that naloxone can displace morphine in the medial thalamic area of the brain after an acute parenteral dose of morphine, suggesting the locus of narcotic receptors in this area. Further work in Way's laboratory [6] showed that less and less naloxone is required to displace morphine in this particular brain area as the degree of dependence is enhanced, which again indicates the correlation between increased naloxone sensitivity and the degree of dependence.

When animals are made highly tolerant by subcutaneous implantation of morphine pellets, the apparent pA_2 of morphine–naloxone increased further such that the affinity constant was now enhanced by nearly 8-fold over the normal value. This indicated that morphine caused a qualitative change in the narcotic receptors with the development of narcotic tolerance. The change is initiated by a single exposure to narcotic drugs, and the receptors continue to change as long as narcotic drugs are available in the milieu. The alteration in receptors stabilizes when the animals become highly tolerant to narcotic drugs. These findings argue against theories of tolerance which infer changes in the number of pharmacologic receptors

or silent receptors. If the change in receptors due to narcotic tolerance and dependence were a quantitative rather than a qualitative one, no changes in the apparent pA_2 should have been observed.

While Herz's group [6] obtained the same apparent pA_2 value *in vivo* for morphine–naloxone as ours of about 7·0, using rats and a different analgesic assay, they were unable to observe an increase in the value in morphine-dependent rats. However, Way's laboratory has been able to confirm both the apparent pA_2 value and the increase in this value in morphine-pretreated and morphine-dependent animals using mice and the tail-flick assay (personal communication).

SUBSTANCES WHICH INHIBIT OR ENHANCE THE DEVELOPMENT OF MORPHINE TOLERANCE AND DEPENDENCE

This topic has been covered thoroughly in recent reviews [1, 18, 19, 21]. A variety of compounds has been shown to modify the development of tolerance and physical dependence; however, in assessing the effect of these compounds, one must initially scrutinize the experimental procedure used by the various investigators. Since the degree of physical dependence is estimated by the severity of the withdrawal signs, one must be careful that the effect of modifying compounds on the development of dependence is distinguished from that on the expression of withdrawal signs. Compounds which are administered to the experimental animals before exposure to the narcotic drug and throughout the course of chronic narcotic treatment should be considered to modify the development of dependence. Compounds which are administered prior to either abrupt withdrawal from chronic narcotic administration, or injection of a narcotic antagonist to acutely precipitate withdrawal, should be considered to modify the withdrawal signs. The investigator should also be aware of compounds which might alter the behaviour of animals to an adverse stimulus when the degree of analgesia and analgesic tolerance is tested. Collier *et al.* [22] have emphasized that an important factor in determining the effect of modifying compounds on withdrawal signs is the time when the compound is administered in the course of dependence induction and withdrawal.

Inhibitors of protein synthesis which have been shown to inhibit the development of tolerance and physical dependence have been compiled [1], and they include agents affecting the processes of transcription, nucleic acid synthesis and translation. Earlier efforts to detect increased amounts or rate of synthesis of proteins or RNA in brains of morphine-tolerant animals have failed. Recently, Loh's group [5] have shown, by measuring UTP incorporation into DNA, that the chromatin template activity in brains of morphine-tolerant animals is significantly increased over that of non-tolerant animals. This effect appeared to be narcotic-specific, since it could be blocked by naloxone. It has been shown with one of the protein synthesis inhibitors, cycloheximide, that it can block the development of tolerance to morphine at a dose that does not affect the acute responses to morphine. Way [18, 19] has interpreted this to mean that the protein concerned with tolerance and dependence is not the morphine receptor but some other macromolecule with a higher turnover rate.

Depletors of the central stores of norepinephrine (NE), dopamine (DA) and 5-hydroxytryptamine (5-HT) appear to inhibit the development of tolerance and physical dependence in rodents. Way [18, 19] believes that 5-HT may be more intimately involved in the adaptive processes than either NE or DA. The pros and cons of this view have been aired elsewhere [1, 18, 19, 21]. The observance that physostigmine and diisopropylfluorophosphate do not materially affect the development of tolerance and dependence led Way [18, 19] to conclude that cholinergic mechanisms do not play a primary role in the adaptive processes. Of recent interest with regard to the cholinergic system is the finding in Dewey's laboratory [6, 23] that intracerebroventricularly applied acetylcholine (ACh) produces analgesia in mice which is blocked by several narcotic antagonists. Curiously, the active narcotic antagonists, *l*-pentazocine and *l*-cyclazocine, do not block the analgesia produced by ACh, whereas the inactive isomers, *d*-pentazocine and *d*-cyclazocine, do block ACh-induced analgesia. Chronic administration of ACh leads to tolerance to the analgesic effect of ACh but not to that of morphine. However, morphine-tolerant mice display cross-tolerance to the effects of ACh. They have not studied the effect of ACh on physical dependence as yet, but further work on these interesting findings should be forthcoming shortly.

Much effort has been expended in studying the inhibition of tolerance and dependence, and rightly so from a clinical viewpoint; however, from the aspect of understanding the basic mechanisms, a remarkable finding by Way's group [18, 19] is that morphine tolerance and dependence in mice can be actually enhanced by certain compounds. So far, the acceleration of tolerance and dependence has been accomplished by three agents, tryptophan, cAMP and γ-aminobutyric acid (GABA). In addition, this enhancement of tolerance and dependence can be blocked by agents which antagonize the respective actions of each of the three enhancing compounds, i.e. by *p*-chlorophenylalanine (PCPA), a depletor of 5-HT, by β-adrenergic blockers which are thought to be inhibitors of cAMP synthesis and by bicuculline, a GABA antagonist. The inhibition of dependence by PCPA has been challenged by several laboratories, and Way [18, 19] has discussed the differences in methodology as the probable reason for the lack of corroboration.

Way [18, 19] has suggested that the accelerating effect of both cAMP and tryptophan on tolerance and dependence may be related to the enhancement of 5-HT turnover. It may be relevant that morphine has been demonstrated to block the stimulation of cAMP formation by prostaglandins E_1 and E_2 in homogenates of rat brain [24] and in neuroblastoma and neuroblastoma X glioma hybrid cells [25]. Morphine also inhibits adenyl cyclase activity of neuroblastoma X glioma hybrid cells stereospecificially, and the inhibition is reversed by naloxone [26]. Collier and Roy [24] proposed that opiates may act on humoral messengers such as the prostaglandin-cAMP system, and tolerance and dependence may represent a compensating hypertrophy of the inhibited prostaglandin-

cAMP system. It is unclear at present how GABA, a putative inhibitory neurotransmitter, fits into the puzzle of accelerated tolerance and dependence.

One of the important advances made in this area is the finding by Way's group [18, 19] that the acute analgesic effects of morphine can be altered with various substances without any change in the development of tolerance and physical dependence and vice versa. For example, adrenalectomy and cholinesterase inhibitors increase morphine analgesia in mice but do not materially affect the development of tolerance and physical dependence. Hemicholinium-3 and 6-hydroxydopamine decrease morphine analgesia but again have no effect on the development of tolerance and dependence. Some compounds which decrease morphine analgesia, such as cAMP and γ-aminobutyric acid, tend to accelerate the development of tolerance and physical dependence, while other agents which have no effect on the analgesic effect, such as propranolol and 5,6-dihydroxytryptamine, tend to decrease the two phenomena. This dissociation of the acute effects of narcotics from the processes of tolerance and physical dependence should be given serious thought with regard to receptor mechanisms.

To throw caution to the wind, it should be indicated that many of the compounds purported to modify the development of tolerance and physical dependence also alter the expression of withdrawal signs in narcotic-dependent animals. Many of these compounds have been tabulated recently [1]. Compounds that attenuate withdrawal signs in various species include modifiers of central biogenic amines, amino acids and derivatives, adrenergic blockers and a number of miscellaneous compounds. Compounds which exacerbate withdrawal signs include predominantly modifiers of central biogenic amines. To complicate matters, some agents that attenuate withdrawal signs in one species may exacerbate them in another species. Although all the biogenic amines appear to be involved in the expression of withdrawal signs, the integrity of the central stores of catecholamines, especially DA, appears to be required for the full expression of abstinence syndrome in morphine-dependent mice, rats, monkeys and possibly man [1]. If certain compounds appear to modify the development of dependence, but also markedly alter withdrawal signs, the assessment of the data becomes difficult, and conclusions as to effects on development of the adaptive process must be drawn with caution.

CONCLUDING REMARKS

Isolation of narcotic or opiate receptors has yet to be accomplished. The opiate binding material from brain fulfills many of the criteria for receptors such as stereospecificity, binding constants in pharmacological concentrations, saturability, reversibility and organ or cell specificity. However, no definitive information that relates alterations in opiate binding to the tolerance–dependence state has been obtained, i.e. when animals are tolerant and dependent on narcotics, they become insensitive to narcotics and at the same time become very much more sensitive to narcotic antagonists, and corresponding changes in opiate binding material have not been observed. Also, the number of types of opiate receptors has not been established. Is there only one kind of opiate receptor or are there different receptors mediating the various actions of narcotics? Do the receptors exist in several conformational states and do they have several different binding sites? Finally, whether the binding of narcotics to the putative opiate receptor material initiates a pharmacological response remains to be seen.

The isolation of "endogenous ligands" for the opiate receptors would no doubt help to enhance our knowledge of narcotic receptor biochemistry; however, one must now muse about not only why opiate receptors in our bodies, but also why opiate-like ligands?

The present evidence implicates protein synthesis and central biogenic amines in the phenomena of tolerance and physical dependence. The biogenic amines appear to play a major role in the expression of the withdrawal signs. Since the acute effects of morphine can be dissociated from the process of tolerance and dependence, and these adaptive processes can not only be inhibited but also accelerated with certain compounds, promotion of newly designed experiments should be encouraged in the hope of elucidating the mechanism of these very perplexing phenomena of narcotic tolerance and physical dependence. Some areas of research such as a number of structure–activity studies and studies on morphine antibodies have not been covered in this short commentary, but results from such studies should also contribute to the understanding of the two adaptive phenomena.

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