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PRELIMINARY COMMUNICATIONS

A STUDY OF DEOXYRIBONUCLEIC ACID BINDING OF NARCOTIC ANALGESICS

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In order to elucidate the mechanism of drug action, it is necessary to identify specific sites of action and to isolate and characterize the drug receptors. The receptor is that specific macromolecule with which a drug binds and initiates the ultimate pharmacologic action of that drug. In recent years, the search for narcotic receptors has been most vigorous. Using radioactive narcotics with high specific activities, several laboratories have demonstrated the possible existence of opiate receptor in the brain homogenate [1-4]. Recently, Lowney et al. [5] have also reported the identification and partial purification of opiate receptor from mouse brain. Loh et al. [6] have presented evidence that the main component in the opiate receptor fraction may be cerebroside sulfate. Navon and Lajtha [7] have shown that brain nuclei and mitochondria can accumulate morphine in vitro and that accumulation can be inhibited by levallorphan and nalorphine.

Since we have reported that chronic morphine treatment results in an increase in chromatin template activity in tolerant mice [8], it is, therefore, important to study the possible binding of narcotics to naked DNA.

The binding of narcotics with DNA was measured according to the method described by Hummel and Dreyer [9]. Sephadex-50 (1 x 8 cm) columns are equilibrated with 1 nM morphine-^{3}H (12.5 Ci/m-mole, New England Nuclear Corp.). DNA (calf thymus), 20 ng dissolved in 1 nM morphine-^{3}H in 0.1 ml solution is applied directly onto the column. The same 1 nM morphine-^{3}H solution is used to elute the column. When 50 per cent inhibition by other analogs is determined, the column is equilibrated with 1 nM morphine-^{3}H and nonradioactive narcotic, and eluted with the same solution. The effluent is collected in a fraction collector for radioactivity measurement. As the DNA-morphine complex peak emerges at the excluded volume of the column, the total amount of radioactive morphine rises above the equilibrium level. Therefore, the total binding of morphine-^{3}H by DNA can be calculated on the basis of the counts.

A typical column pattern with 200 μg DNA and 8 nM morphine-^{3}H is shown in Fig. 1. The effluent solution was measured with a Beckman DBG spectrophotometer set at 260 nm, when 200 μg

or more DNA was used. However, in later experiments, when 20 ng DNA was the choice, the absorbance at 260 nm was not followed.

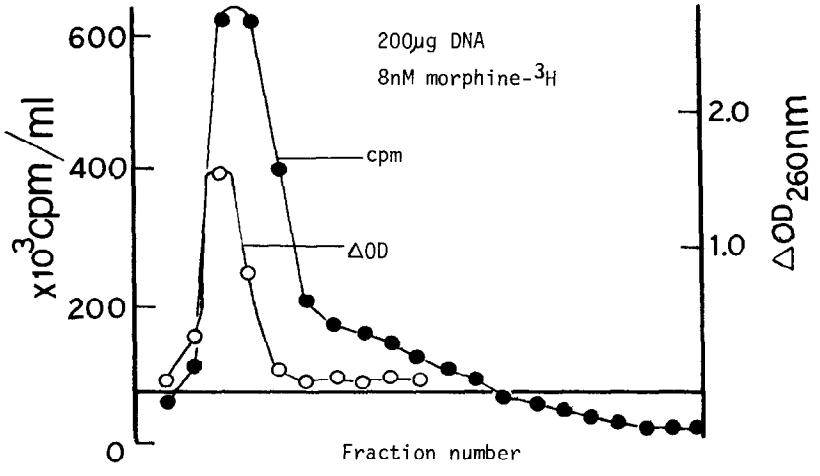

Fig. 1. Typical elution pattern of DNA-morphine binding. The fraction size is 1 ml. The column is prepared as described in the text.

The binding of morphine to DNA is saturable; the dissociation constant is 1.5×10^{-8} M, calculated by the method of Scatchard [10]. Other agonists or antagonists can inhibit the binding of DNA and morphine. The concentration of narcotic homologs required to inhibit 50 per cent of 1 nM morphine binding to DNA is shown in Table 1.

TABLE 1. Concentration of some narcotic homologs in preventing morphine-^{3}H binding to DNA

	Concentration required for 50% inhibition (µM)
Naloxone	3.2
Morphine	2.2
Levorphanol	0.2
Dextrorphan	2.4

Naloxone and morphine are comparable as inhibitors of the binding of DNA and morphine. Levorphanol is about 8 times more potent than its optical isomer, dextrorphan. Thus, the binding is stereospecific. Separate experiments using ^{3}H-dextrorphan and ^{3}H-levorphanol (10^{-7} M, donated by Hoffmann-La Roche) also showed that the stereospecific binding is 82 per cent.

Navon and Lajtha [7] have reported that the brain nuclei can actively take in

narcotic, suggesting that nuclei may play a possible role in morphine action.

The present study demonstrated that thymus DNA can, indeed, bind morphine with high affinity. The dissociation constant, 1.5×10^{-8} M, is in the same concentration range reported by many investigators for narcotic receptors [1,3]. Stereospecificity is also demonstrated. The binding of morphine to DNA can be inhibited by nonradioactive morphine, naloxone, levorphanol or dextrorphan. All the data presented above suggest that DNA may be one of the narcotic receptors. It is unlikely that one would conclude that thymus DNA is, indeed, responsible for analgesia, or even for the development of tolerance and physical dependence induced by morphine, since it is believed that the brain is the primary target system for narcotic action. However, it is generally accepted that all cells of the higher organisms contain the same genetic information and that differentiation is based upon the selective expression of different parts of that information. To be more specific, the selective expression of different parts of the gene lies in the specific interaction of nucleoproteins and naked DNA. The study of DNA and narcotics in the presence of different nucleoprotein isolated from the brain would yield more critical information regarding the mechanism of narcotic action. However, one may not ignore the fact that morphine may, indeed, interact with the genetic material and the interaction is specific and selective.

The present report raises the possibility that morphine may have an effect on brain nuclei, and that the interaction may be most interesting. Investigation in this laboratory has revealed that chronic morphine treatment increases the chromatin template in directing UTP incorporation. This phenomenon may result from morphine-DNA interaction. The exact relationship between pharmacological action and the specific binding remains to be elucidated.

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