totherapy could be peculiar to a developing neuroendocrine system and might have little similarity to results that would be derived from adult organisms.

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Opiate Receptors for Behavioral Analgesia Resemble Those Related to the Depression of Spinal Nociceptive Neurons

Abstract. *With naloxone as antagonist, a dose-ratio analysis of the depression by morphine of nociceptive neurons in the spinal cord reveals that this opiate depression of single unit activity has the same pharmacological properties as observed with morphine analgesia. This suggests that the opiate receptor, mediating the observed cellular depression, and those mediating analgesia are presumably the same.*

Lamina 5 neurons in the dorsal horn of the spinal cord are discharged by the administration of noxious stimuli to their receptive fields and by Aδ and C fiber activation, while the application of narcotics in doses sufficient to produce analgesia will depress this discharge (*1, 2*). If these cells are in fact part of the pathway through which behaviorally defined changes in the pain threshold are mediated, then the pharmacology of the opiate effect measured on the cellular response should be identical to the pharmacology of the analgesia resulting from these drugs. Both the neuronal activity of these cells and the behavioral relief from pain produced by opiates are stereospecific and antagonized by naloxone (*1–3*). However, the simple observation of naloxone antagonism is not a sufficient premise to assume the pharmacological identity of the receptor systems mediating either effect. The dose ratio analysis (*4, 5*) in the behaving animal has demonstrated that different values for the pA_2, a parameter reflecting the interaction between a competitive antagonist and agonist in a given receptor system, may be obtained for morphine and naloxone when different opiate effects, such as respiratory depression, temperature, and analgesia, are measured (*6*). Such results suggest that different forms of the opiate receptor may be related to brain structures mediating these various functions. If the depressive effects of systemically administered opiates on the response of lamina 5 neurons to noxious stimuli are related to the substrate mediating the observed behavioral analgesia, then the dose ratio analysis carried out with the discharge of the cell rather than the behavioral response as an end point should yield the same results, as has been reported for the interaction of morphine and naloxone on analgetic tasks in the behaving animal.

These studies were conducted as follows. Microelectrode penetrations (*7*) were made into the dorsal horn of the unanesthetized, decerebrate spinal cat which had been paralyzed with flaxedil and artificially respirated. During penetration, the sural nerve, mounted on silver hooks and cut distally, was stimulated at an intensity which produced an Aδ and C volley as monitored on adjacent recording hooks. Cells were selected for study only if they showed a relatively low level of background activity (2 to 10 Hz) and if they responded to sural nerve stimulation with a stable discharge pattern having an identifiable fast and slow component corresponding to the large and small fiber input. The amplified response of the cell went into a spike trigger, and this output went both into a rate meter (spikes per second) and into an averager to give a post-stimulus histogram (PST). Throughout the experiments, the shape of the spike was continuously monitored by a delay line to verify that the recorded activity was derived from a single isolated cell and that the same spike was present during the entire experimental sequence. One of two drug paradigms was used. In the first, morphine was given in two doses; the second dose was ten times larger and was delivered after the first dose had produced its maximum effect. In this manner, a dose response function was obtained for each unit. Three different morphine dose ranges were used to give a dose response curve ranging from 0.3 to 10.0 mg/kg (that is, 0.3 and 3.0, 0.5 and 5.0, and 1.0 and 10.0 mg/kg). As the dose separation was always a factor of 10, the effect of the first dose on the effect produced by the second dose was considered negligible. At the time that the effect of the

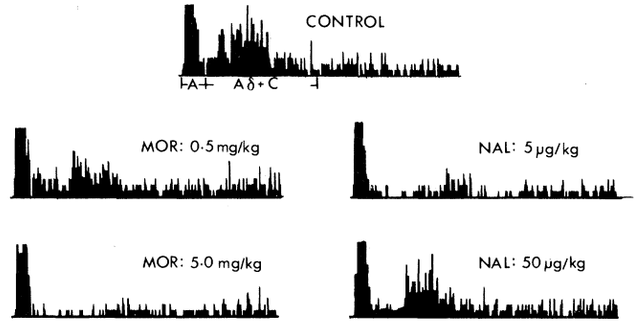

Fig. 1. Post-stimulus histograms each indicating the average of 32 sweeps and derived from a single cell before (*CONTROL*) and after the injection of morphine sulfate (*MOR*) and naloxone hydrochloride (*NAL*). The sequence of injections was morphine (0.5 mg/kg) followed by morphine (5.0 mg/kg). At this time, naloxone was given at 5 μg/kg and then 50 μg/kg. Ten minutes elapsed after each injection of morphine and 5 minutes after each naloxone injection. The evoked discharge related to large (*A*) and small (*Aδ and C*) fibers is indicated in the control PST. The axis bars are 100 msec and 20 spikes. As the settings required to examine Aδ and C fiber evoked activity, the response to A fibers exceeded the capacity of the averager, resulting in the sharp cut off seen in these records.

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second dose of morphine was at the maximum, naloxone was injected in two doses that were separated by a factor of 10, with the second dose of naloxone being given when the antagonistic effects of the first naloxone dose were observed to be maximal (*8*). In a number of experiments, morphine was given in a single dose, and the effect of a single dose of naloxone was observed. This conventional approach permitted me to determine whether the results were being biased by some unforeseen multiple dose interaction.

Figure 1 presents the PST of a cell for which the effects of morphine in two sequential doses (0.5 and 5.0 mg/kg) were examined. This is followed, in the manner described above, by two doses of naloxone (5 and 50 μg/kg). The dose-dependent depression of the late Aδ and C fiber mediated discharges by morphine and its antagonism by naloxone can be seen. In Fig. 2a, the dose response data for six cells are presented. Naloxone over the dose range of 5 to 100 μg/kg produced a parallel shift to the right in the morphine dose response curves, with the effective dose of morphine required to produce a 50 percent inhibition (ID_{50}) going from 1.4 mg/kg without naloxone to 11.1 mg/kg with the highest dose of the antagonist (100 μg/kg). In calculating these figures, the level of spontaneous activity at each dose was subtracted from the level of evoked activity. These curves therefore represent the effect of morphine on the stimulus-evoked activity. Although not presented here, the effects of a single dose of morphine over a dose range of 0.5 to 5.0 mg/kg antagonized by single doses of naloxone (10 or 50 μg/kg) were examined in 19 other cells having similar response characteristics. The ID_{50} obtained for morphine under these conditions was 1.6 mg/kg. When naloxone was administered at 5 or 50 μg/kg, respectively, the ID_{50} for morphine was 2.1 and 7.2 mg/kg. These values correspond with the results presented in Fig. 2a and validate the data obtained with the multiple dose paradigm. Figure 2b is a plot of the dose ratio obtained from these dose response experiments. The four points describe a best-fit line with a slope close to −1 (−0.92) and from which we can extrapolate a pA_2 of 7.23.

These observations correlate well with the pA_2 values obtained for the interaction of naloxone with morphine on a variety of analgetic tests ranging from the tail flick to the shock titration paradigm in which rats and primates were used. In all cases, the pA_2 across this diversity of species is about 7 (*4, 5*). This value is also similar to that obtained for naloxone in the rat when analgesia was produced by the intrathecal injection of either morphine or several metabolically protected pentapeptides (*9*). This consistency of the pA_2 suggests that the receptor interaction reflecting opiate action is the same, not only across species but the same for analgetic tasks having both spinal and supraspinal components. This consistency, moreover, contrasts with the findings mentioned previously that pA_2's obtained for naloxone on other opiate sensitive responses differ. This general observation suggests that other sites of opiate action associated with other physiological systems may have pharmacologically different characteristics.

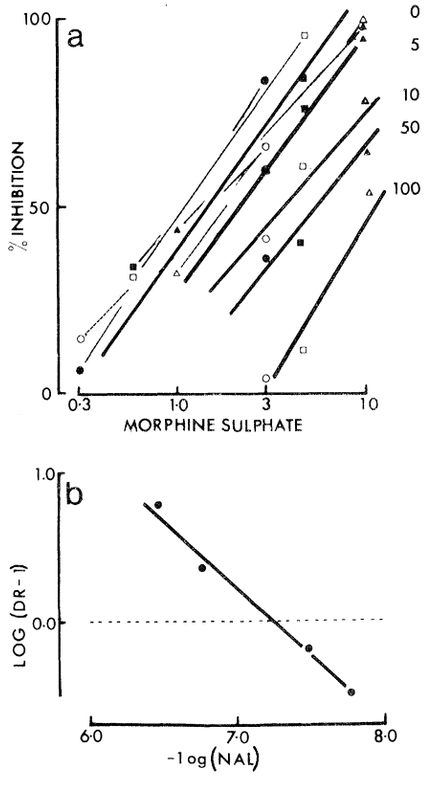

Fig. 2. (a) Log-dose response curve for showing inhibition produced by morphine (as sulfate, in milligrams per kilogram) as a function of increasing dose of naloxone (0, 5, 10, 50, and 100 μg/kg). Inhibition is expressed as the percentage decrease in the magnitude of the Aδ and C fiber discharge as a function of the control prior to administration of the drug. All percentages are based on evoked activity alone. The heavy black line indicates the best-fit line for the associated points. The light dotted line connects those points obtained in each cell prior to the injection of naloxone. Each symbol represents the data from a single cell derived as shown in Fig. 1. (b) Dose ratio plot derived from the dose response data presented in (a) ($DR - 1$) is explained in (*4*). The values for $-log\ (NAL)$ are moles per kilogram.

Several workers have in fact suggested the likelihood that different forms of the opiate receptor may exist (*10*), and that the different forms may be associated with different physiological systems. The data presented, however, show that the system of which the lamina 5 interneuron is a part is affected by the opiate sensitive system in the spinal cord in the same manner as the functionally defined system that mediates the behavioral phenomena of analgesia produced by morphine. These results do not say that the opiate synapse is on the lamina 5 neuron although the use of the spinal animal precludes any opiate effect originating from supraspinal structures. In view of the fact that opiate binding is the highest in the substantia gelatinosa (*11*), it would appear that the terminals of the opiate sensitive system in the cord may be there. The input to the deeper interneurons might thus be mediated by some neuron whose activity is reflected in part by opiate action in these more dorsal laminae. Evidence for this thinking derives not only from the binding data, but also from work in which the iontophoretic administration of opiates into the substantia gelatinosa has been shown to depress the activity evoked in lamina 5 neurons (*12*) by nociceptive stimuli.

Aside from the importance of the above observations regarding the interaction of opiates with respect to sensory input, the experiments demonstrate the utility of the dose ratio analysis in studying drug effects on single cell activity. The use of this pharmacological approach in examining the receptor systems associated with specific neuronal systems would appear to have importance for understanding of other complex behavioral phenomena.

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4. A compound that is a competetive antagonist of a given receptor system will produce a parallel shift of the agonist dose response curve, the magnitude of the shift being proportional to the log dose of the antagonist. A plot of $-\log (DR - 1)$—where DR is the dose of the agonist required to produce a given effect in the presence of a given dose of the antagonist divided by the dose of the agonist required to produce the same effect without the antagonist—as a function of −log (antagonist, in moles per kilogram) yields a line whose intersection with the abscissa is the pA_2, that is the negative log of the

dose of the antagonist in moles per kilogram which will produce a doubling of the dose of the agonist required to produce the desired effect. The assumptions underlying the use of this procedure in vivo have been discussed (5). [See also A. E. Takemori, *Adv. Biochem. Psychopharmacol.* **8**, 335 (1974)].

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8. The maximum degree of morphine depression of cell discharge was usually reached within 4 to 8 minutes, and the depression remained stable for approximately 20 to 30 minutes, depending on the dose. For this reason, a standard interval of 10 minutes between morphine doses was consistently employed. Naloxone, being more lipid soluble, has a more rapid onset and produced a maximum degree of antagonism after 3 to 4 minutes. Naloxone injections were therefore separated by 5 minutes. In previous experiments (2), naloxone at the highest dose used in these experiments (100 μg/kg) was observed to have none or only a minor effect on either the spontaneous activity or the magnitude of the Aδ and C fiber–evoked component. The effect was variable and not associated with any particular population of cells.
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13. These experiments were carried out while visiting with Professor P. D. Wall at University College, London. I thank J. O'Connor, A. Ainsworth, and J. T. Patel for their technical assistance.

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Cardiac Pacemaking

The idea of Pollack (*1*) that sinus cells release catecholamines, thereby initiating and maintaining pacemaker activity, is not supported by experimental evidence. Two necessary steps in the sequence of events that keeps sinus cells firing are, according to Pollack: (i) interaction of catecholamines with sinoatrial β receptors and (ii) stimulation of the adenylyl cyclase. For (i) he quotes the experimental finding that a $10^{-5}M$ concentration of the β-receptor antagonist propranolol causes modest negative chronotropic effects in the neonatal mouse and arrest of cultured beating cardiocytes. From published experiments (*2*) it can be calculated that as little as $3 \times 10^{-9}M$ concentrations of racemic propranolol occupies 50 percent of sinoatrial β receptors stimulated by catecholamines. Similarly, $3 \times 10^{-9}M$ propranolol produces 50 percent occupancy of β receptors coupled to the adenylyl cyclase of cardiac membranes (*3*, *4*). However, $3 \times 10^{-9}M$ to $1 \times 10^{-6}M$ propranolol does not slow sinoatrial beating (*2*). Exposures of right atria to $10^{-6}M$ propranolol causes 99.7 percent β-receptor occupancy and requires a 300-fold increase of catecholamine concentration to achieve the same positive chronotropic effect as in the absence of propranolol. Thus, if the catecholamine release postulated by Pollack maintains beating rate, the sinoatrial cells in $10^{-6}M$ propranolol should release 300 times more catecholamine to keep their beating rate undiminished. This would imply an extraordinary adaptability of the pacemaker cell in furnishing the precise amount of catecholamine required to keep beating rate constant. It would also mean that such adaptability should be nearly instantaneous, because no fast transient decrease and reestablishment of beating frequency is observed with the administration of propranolol at a concentration of $10^{-6}M$. The depression or suppression of beating rate caused by the very high concentration ($10^{-5}M$) of propranolol quoted by Pollack is merely due to a toxic effect (*5*), unrelated to the drug–β-receptor interaction. Another high-affinity β-receptor antagonist, (−)-bupranolol (KL 255) (*3*) does not cause significant changes of beating frequency in cultured myoblasts (*6*) and right atria (*7*) from rats, while yielding 98.0 and 99.9 percent β-receptor occupancy, respectively. The commented evidence with (±)-propranolol and (−)-bupranolol strongly supports the view that sinoatrial and myoblastic cells can generate spontaneous beating activity when their β-receptor–catecholamine interaction is prevented. This is out of line with Pollack's suggestion of an obligatory role of sinoatrial catecholamines for eliciting automatism. Besides, an obligatory role of catecholamines for myocardial pacing would be catastrophic to the numerous patients who receive propranolol and other β-receptor antagonists as treatments for various cardiovascular diseases.

One should be cautious about accepting the contention of some authors quoted by Pollack that the beating rate of cultured cardiocytes under the influence of cardioactive drugs is directly proportional to the cellular concentration of adenosine 3′,5′-monophosphate (cyclic AMP). For instance, while submaximally effective concentrations of catechols cause an equilibrium increase of beating rate in myoblasts, their cellular content of cyclic AMP increases and quickly decreases again during steady state chronotropic effects (*7*). This evidence is inconsistent with a simple causal relationship between levels of cellular cyclic AMP and positive chronotropic effects of catecholamines.

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19 May 1977

Pollack (*1*) favors the view that catecholamines have an obligatory role in cardiac pacemaker activity. He bases this largely on the fact that isolated hearts or cultured heart cells cease to beat after treatment with reserpine. He does not review numerous articles which describe effects on heart rate of full blocking doses of β-adrenergic blocking agents, ganglionic blocking agents, or of atropine, given alone or in combination, to intact animals.

When the mammalian heart in situ, which is exposed to many substances not present in solutions used to support isolated tissues, is made unresponsive to injected catecholamines (and to acetylcholine) by blocking agents, the rate becomes quite constant. Cardiac arrest, or even bradycardia, does not occur. Pollack could say that although effects of exogenous catecholamines are blocked, endogenous catecholamine still is needed to maintain the heart beat. However, he theorizes that the endogenous catecholamine is passed out of the cell by exocytosis and then it exerts its action. It seems that a β-adrenergic blocking agent that would prevent the action of a substance arriving by the blood stream would also block the action of the

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