published work. The minimonster created is that in the minds of some, the second most prestigious position today for listing one's name on a multiauthored publication is now neither second nor last but rather third. This, along with hula hoops and pet rocks, will be difficult to explain to the next generation.

Whether these small changes can be considered progress is debatable, rather they are made in the interest of uniformity and common sense. If those interests are served, our purpose is accomplished.

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Opiate Receptors and Their Definition by Antagonists

THAT RECEPTORS EXIST as a physical entity is in no doubt. Nevertheless, the term "receptor" more often than not is a hypothetical construct used to describe drug effects vis-a-vis a given physiologic endpoint. As such, the receptor represents an operationally defined entity through which agents are able to exert a powerful effect on cellular function.

The relative potency of a series of structurally related agonists which produce a given physiologic or biochemical effect define a structure-activity relationship that is *characteristic* for that receptor. Thus, a receptor is defined in the same way one would define a lock by the set of keys that operate it. A particularly valuable paradigm for studying receptors has been the development of agents with high affinity for a particular receptor and low efficacy, *i.e.*, antagonists. Thus, an antagonist will recognize a given receptor conformation and, while occupying that receptor, prevent activation by an agonist that would otherwise act on that receptor. Thus, it is possible in a shorthand fashion to "define" the nature of the receptor with which some novel agonist interacts by determining whether a receptor-selective antagonist is able to effectively block the effects produced by the novel agonist.

Receptor interactions of particular agonists and antagonists are characterized by certain quantifiable parameters; one of these is the pA_2. *In vitro*, the pA_2 is the negative log of the concentration of antagonist that doubles the concentration of agonist necessary to produce a particular level of response. If experimental conditions are appropriate, the pA_2 is the negative log of the antagonist-receptor equilibrium dissociation constant.[1] If a series of agonists act on the same receptor, then, though different concentrations of each agonist may be required to produce the *same* physiologic or biochemical effect, the pA_2 of the antagonist will be the same no matter which of the agonists is employed to determine it.

In vivo, dose must be substituted for concentration since it is usually not possible to adequately determine the tissue concentration of antagonist and, therefore, the pA_2 can no longer be strictly identified with the negative log of the dissociation constant. However, it is often reasonable to assume that dose is proportional to concentration, and the criterion that if two agonists are acting on the same receptor they should yield similar antagonist pA_2 values is still valid.

Studies examining the antagonism by naloxone of the analgesia produced by a wide variety of systemically or intrathecally administered opiate alkaloids and peptides including morphine, levorphanol, ethylketocyclazocine, [d-ala^2-met^5]-enkephalin, and β-endorphin, have uniformly provided pA_2 values of approximately 7.[2,3] That the naloxone pA_2 obtained in the presence of these structurally diverse agonists is the same suggests that each of these agonists is producing its effects by an action on the same receptor. In contrast, the pA_2 value of naloxone obtained in the presence of [d-ala^2-d-leu^5]-enkephalin is approximately 6, suggesting that naloxone has a lesser affinity for the spinal receptors acted upon by this peptide to produce analgesia.[4]

Further definition of a receptor population can be achieved by comparing the affinities of a number of antagonists for a pharmacologically similar population of receptors. If the same receptor population is acted upon by a series of agonists, then the relative ordering of the pA_2 values for the several antagonists vis-a-vis these different agonists should be the same. Thus, the pA_2 of naltrexone in the presence of morphine is 8.[5] It would be predicted that those agonists for which naloxone has

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a pA_2 value of 7 (*e.g.*, morphine, β-endorphin, *etc.*) would also give a naltrexone pA_2 of 8. Thus, while naltrexone is 10 times more active than naloxone, the similarity of the pA_2 across different agonists for naloxone *and* naltrexone strengthens our concept that, in fact, these two antagonists and the several agonists for which the pA_2s are homogeneous may in fact act upon the same population of receptors. The more times we repeat this paradigm with antagonists of different affinity and different agonists the more secure we can be that the several different ligands may be exerting their physiologic effect on the same receptor population and that the receptor population mediating a given physiologic endpoint is homogeneous.

In short, the receptor(s) mediating a given physiologic or biochemical endpoint is defined by several qualitative and quantitative descriptors, *e.g.*, agonist structure-activity relationship, antagonist structure-activity relationship, and the quantitative interaction between agonists and antagonists.

This discussion emphasizes that it is not adequate to show that a selective antagonist blocks an effect of another drug to support the claim that the agents work on a given receptor. The blockade must obey certain characteristics. The simplest characteristic is that the antagonist's inhibition of the effect produced by a given agonist occur within the limits indicated by the pA_2 value. Thus, if an agent acts through an opiate receptor, as defined by any of the structure-activity series thus far presented, the doubling of the ED_{50} of the agent by naloxone will occur at naloxone doses ranging somewhere between 30 and 300 μg/kg. If significantly higher doses are required, then in fact there may be alternate interpretations. First, it may be that such high doses reveal low affinity interactions of naloxone at "opiate" receptors other than the mu and delta opiate receptor subtypes for which it has demonstrated very high affinity. This would in fact be a receptor antagonism. However, it is questionable that such a receptor interaction should be designated as opiate in character. In the event that such high doses are required to produce an effect, it becomes necessary to very clearly define the pharmacologic characteristics of the naloxone effect. Thus, it has been suggested, that at high doses naloxone may act as a GABA antagonist. High doses of naloxone produce both biochemical (changes in cerebellar cGMP[6]) and physiologic (seizures and convulsions[7]) effects resembling those produced by GABA antagonists. Moreover, *in vitro* naloxone has been shown to displace ^{3}H-GABA in binding studies.[7] If it is thought that naloxone is producing its effect by an action on receptors whose characteristics resemble those acted upon by naloxone at lower concentrations, this can be substantiated if the activity of other antagonists, *e.g.*, naltrexone, at this receptor also resemble their activities at these other more well-defined receptors. If another structurally different opiate antagonist can be shown to have its traditional spectrum of activity, then it is less likely that the activity of the first antagonist was "non-opiate."

It should be noted that a physiologic antagonism is also possible, *e.g.*, general anesthetics and naloxone may act upon totally different systems whose functions are physiologically opposed. Thus, endorphins and GABA have powerful inhibitory effects on CNS neural activity. Antagonism of their actions by low or high concentrations of naloxone, respectively, might serve to oppose the depressant effects of general anesthetics. Regardless of whether these effects of naloxone are related to an opiate receptor interaction or not, to define the effects of general anesthetics as opiate-like in such a situation is obviously meaningless.

So what? If one wishes to argue that general anesthetics act through an opiate receptor, then the following criteria should be met. 1) The agent should produce physiologic effects that resemble the effects produced by other opiates. 2) The effects produced by the agent should possess a pharmacology characteristic of the activation of opiate receptors. How well then do the general anesthetics fulfill these criteria?

A primary effect of opiates is analgesia. The fact that general anesthetics such as pentobarbital and halothane are thought to be poor analgesics argues strongly against these agents exerting significant effects on opiate receptors. On the other hand, ketamine has been noted for its postoperative pain relieving properties. It is also true that both opiates and general anesthetics have sedating properties. Thus, one presumes that the measured endpoint, *i.e.*, loss of righting reflex as measured by Kraynack and Gintautas,[8] may reflect an outcome common to opiates and general anesthetics.

Though the general anesthetics do not fall within the structural constraints that we have associated with opiates, and therefore do not appear to fall readily within the structure activity series we associate with opiates, it is possible that general anesthetics may act indirectly via an opiate receptor by releasing or facilitating the action of an endogenous agent. However, whether the effect is due to a direct action on opiate receptors or to the action of endogenously released opioids, the physiologic effect produced by the general anesthetic must still meet the pharmacologic criteria that have been established for opiate receptors vis-a-vis well-defined antagonists. In the article appearing in this issue of ANESTHESIOLOGY, Kraynack and Gintautas[8] observed that the systemic administration of naloxone in doses of 50 mg/kg had no effect on the loss of righting reflex produced by these general anesthetics. As noted in the preceding discussion, the *in vivo* pA_2 values indicate that the opiate receptor whose pharmacologic profile was described, is antago-

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nized significantly by naloxone in doses of 0.03 to 0.3 mg/kg, and naltrexone in doses of 0.003 mg/kg. Brain concentrations achieved by the intracerebral injection of 0.25 mg naloxone and naltrexone are no doubt equivalent to even higher systemic doses and even then naltrexone had no effect. Thus, if we assume for simplicity that 50 mg/kg of either drug were administered systemically, doses of 160 to 16,000 times those necessary to significantly antagonize classical opiate effects, did not block the measured action of general anesthetics. As these drugs were administered during times when their peak activity clearly would correspond to that produced by the analgesic drug (as measured by the behavioral endpoint), it does not appear likely that this lack of effectiveness can be attributed to a failure of the drug to reach maximum brain concentrations during the time when the anesthetic effect was evident. As the half-times of naloxone and naltrexone in rat brain are in excess of 30 min, it does appear likely that the agents are present in brain for sufficient periods to alter a behaviorally measurable endpoint.

In short, on the basis of the results of Kraynack and Gintautas[8] one may conclude that the effect on the righting reflex of these anesthetic agents is not mediated by a receptor for which naloxone or naltrexone have an affinity comparable to that of the receptor acted upon by morphine and other alkaloids.

The literature, however, does not permit us to conclude that there is *no* interaction between general anesthetics and opiate sensitive neural systems. Though the literature is controversial, there are numerous reports that naloxone or naltrexone can diminish the effects of nitrous oxide,[9-11] ketamine,[12-15] barbiturates,[16-18] and halothane.[19] Nevertheless, for virtually every anesthetic, there are comparable experiments[20-25] where naloxone failed to have any effect at a variety of doses in a variety of species on the anesthetic or analgesic effects as determined by a number of endpoints. In contrast, no investigator to our knowledge has ever failed to see the analgesic effects of morphine antagonized by naloxone.

Such variability in the results obtained by various investigators may in fact reflect the properties of a physiologic as opposed to a pharmacologic antagonism as suggested by Kraynack and Gintautas in their article[8] and others.[18] Thus, in decerebrate spinal animals in which no anesthesia is employed, the resting and driven activity of some dorsal horn nociceptive neurons and reflexes are enhanced remarkably by doses of naloxone and naltrexone known to be sufficient to antagonize the analgesic effects of morphine.[26-28] It is possible, that this increased activity may reflect the blockade of a tonic inhibition as might be exerted by intrinsic endorphinergic neurons. Alternately, in view of the fact that naloxone has the ability to antagonize GABA, albeit at high doses, this enhancement of dorsal horn neurons responsiveness to noxious stimuli may reflect the loss of tonic GABAergic inhibition.

In short, while we will not rule out the likelihood of a physiologic interaction between opiate antagonists and general anesthetics, at the present time we feel there is no compelling *in vivo* evidence to indicate that the predominant effects of general anesthetics are mediated by a simple action on or through an opiate receptor.

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A Collaborative Clinical Trial on Trial

COLLABORATIVE STUDIES have very high morbidity rates (deviation from the protocol) and very high mortality rates (total lack of success) because some investigators find it difficult to comply with the protocol. Sylvester *et al.* amplify on this problem in their recent critique of the impact of protocol deviation on the success of cooperative trials in cancer therapy.[1] The multi-center study of dantrolene's efficacy in the treatment of malignant hyperthermia published in this issue of ANESTHESIOLOGY[2] is certainly no exception to this conformity problem. The study is in the preterminal morbid state (questionable success): four out of 21 cases of malignant hyperthermia were not treated according to protocol. But this collaborative dantrolene study must be kept alive because the message it whispers (uncontrolled biases sap its strength) is very important clinically; and should it die, it is unlikely that another collaborative study would be attempted.

Could we have expected a more robust study which would have better protocol conformity? It is doubtful that any collaborative study of a very low incident problem could be expected to fare better. Collaborative studies require pilot work in every involved institution to test and clarify the protocol, to train personnel involved in the execution, and to sharpen the focus on reporting and analyses. Unfortunately, the low incidence and high severity of malignant hyperthermia mitigated against the luxury of even one pilot case in each hospital and virtually assured that this multi-institutional study would have potentially fatal nonconformity problems. The investigators are to be complimented for their ability to keep this whispering oracle alive.

What are the biases that weaken the study's claim that patients treated with dantrolene plus symptomatic therapy have significantly lower mortality rates than those treated with symptomatic therapy alone? What are the implications of these uncontrolled biases and how might they have been controlled?

Hospitals and investigators were not selected randomly; it is fairly certain that many volunteered to participate in this study. Was this high level of interest in malignant hyperthermia a factor in determining the results? If so, how would it bias the data? One could speculate that physicians with a keen interest in malignant hyperthermia would be more skilled at diagnosing less severe cases of that disease. Milder cases would be more likely to have spontaneous remissions, or they would be more likely to recover reasonably quickly with aggressive symptomatic therapy which did not include dantrolene. In this nonrandomized study dantrolene was given to everyone diagnosed as suffering from malignant hyperthermia, and those milder cases which would have survived without the use of dantrolene are counted as dantrolene cures. The authors attempted to correct or minimize this bias by eliminating six cases classified *a posteriori* as questionable malignant hyperthermia. The results would have been more convincing had the authors required blindness for this *a posteriori* categorization of patients according to their severity of malignant hyperthermia. Blinding theoretically could have been achieved by eliminating the knowledge of dantrolene treatment

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