Reports

Possible Mechanism of Tolerance to Narcotic Drugs

Although many hypotheses have been offered to explain the development of tolerance to narcotic drugs (*1*), adequate experimental data have not been presented to elucidate this phenomenon. In recent studies at this laboratory, we have observed several striking similarities between the receptors for narcotic drugs and the enzymes that N-demethylate these drugs. The enzymes and receptors have been found to be alike with respect to substrates with which they interact, stereospecificity, and antagonism by N-allylnormorphine (*2*). Since the enzymes that N-demethylate narcotic drugs were similar in several ways to narcotic drug receptors, it appeared likely that these enzymes might serve as a model for the receptors. Thus, any changes occurring in enzyme activity during the development of tolerance might reflect changes taking place on the drug receptor. With this in mind, an examination of the effect of repeated administration of morphine to rats on the enzymic N-demethylation of morphine and other narcotic drugs was undertaken. The effect of the administration of morphine, together with its antagonist, N-allylnormorphine, on enzymic N-demethylation was also investigated, since it has been shown that this combination reduces the development of tolerance (*3*).

Twelve rats were made tolerant to morphine by a daily intraperitoneal injection of morphine sulfate. The animals were given an initial dose of 20 mg/kg of morphine sulfate, and the amount of drug administered was then progressively increased during a period of 35 days until daily injection of 150 mg/kg was reached (group M). Another group of eight rats was given N-allylnormorphine and morphine in a ratio of 1/4 for 35 days (group NM). A group of 12 rats was given the same dosage regimen of morphine as described in group M for 35 days, following which the drug was abruptly withdrawn for 12 days (group W). Fourteen rats receiving a daily injection of isotonic saline served as controls (group C). Fisher-strain male rats that were 120 to 130 days old when the study was begun were used throughout the study. The average gain in weight in all groups of rats was approximately the same.

Twenty-four hours after the test period the animals were sacrificed, and the livers were examined for their ability to N-demethylate morphine, dilaudid, meperidine (Demerol), and cocaine. The livers were prepared for enzyme assay by a procedure described previously (*2*), and the degree of enzymic N-demethylation was determined by estimating the amount of formaldehyde liberated (*4*).

The changes in the enzymic N-demethylation in the various groups of rats are shown in Fig. 1. In the case of morphine-treated animals (group M), a profound reduction in the ability to N-demethylate morphine occurred. In addition, the enzymic N-demethylation of dilaudid, a compound that shows cross-tolerance to morphine (*5*), was reduced to about the same degree as that of morphine, while the demethylation of meperidine, a drug that exhibits limited cross-tolerance to morphine (*6*), was only partially reduced. Enzymic N-demethylation of cocaine, for which no cross-tolerance to morphine occurs (*5*), was unaffected by chronic morphine administration. In the group of animals that was treated with both N-allylnormorphine and morphine (group NM), the reduction in the enzymic demethylation of narcotic drugs was significantly less than in those that received morphine only. The enzyme activity with respect to all substrates had returned to the control level or above in withdrawn animals (group W).

Other pathways in the *in vitro* metabolism of narcotic drugs, such as O-demethylation of codeine (*7*), hydrolysis of diacetyl morphine (*8*), and conjugation of morphine (*9*), were also examined. No differences in the enzymic O-demethylation, hydrolysis, and conjugation of narcotic drugs in the control- and morphine-treated rats were found.

From the results described here, a striking parallelism between the enzymic N-demethylation of narcotic drugs and the development of tolerance to these drugs was found. The repeated administration of morphine reduced both enzymic demethylation and pharmacological response. In addition, there was a correlation between demethylation of substrates and cross-tolerance to morphine. Furthermore, N-allylnormorphine, which blocks development of tolerance to morphine, also blocks reduction of enzyme activity. It appears that N-allylnormorphine not only antagonizes the pharmacological action and the enzymic demethylation of narcotic drugs but also protects the enzyme and perhaps the receptor sites. Animals that are withdrawn from narcotic drugs recover their pharmacological responses to these drugs; similarly, the demethylating-enzyme activity in rats withdrawn from morphine returns to normal.

The changes in enzyme activity in morphine-treated rats suggest a mechanism for the development of tolerance, if one assumes that enzymes which N-demethylate narcotic drugs and the receptors for these drugs are probably closely related. The continuous interaction of narcotic drugs with the demethylating enzymes inactivates the enzymes. Likewise, the

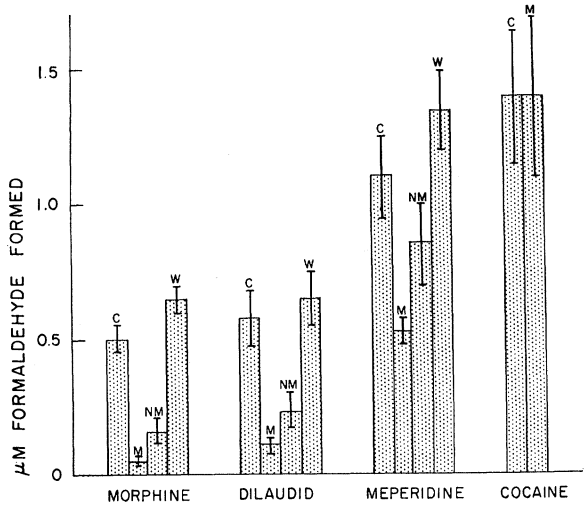

Fig. 1. Effect of morphine treatment, N-allylnormorphine, and withdrawal on the enzymic N-demethylation of narcotic drugs. Vertical bracketed lines on bars are standard deviation of the mean. (group M) morphine-treated rats; (group NM) morphine- and N-allylnormorphine-treated rats; (group W) rats treated with morphine and then withdrawn; (group C) normal rats.

continuous interaction of narcotic drugs with their receptors may inactivate the receptors. Thus, a decreased response to the narcotic drugs may develop as a result of unavailability of receptor sites (*10*).

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References and Note

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Apomorphine Test for Tranquilizing Drugs: Effect of Dibenamine

When a minimal emetic dose of apomorphine is carefully established in a group of dogs, it is possible to detect the inhibiting effect on emesis of a second drug, such as diphenhydramine (*1*), chlorpromazine (*2*), or reserpine (*3*). These findings indicate that the apomorphine test may have utility in selecting tranquilizing agents and imply a link between central emetic mechanisms and activities effecting tranquil behavior.

The rationale for the apomorphine test lies in the drug's established site of action at the chemoreceptor trigger zone in the area postrema, an area afferent to the emetic center which lies more deeply in the lateral reticular formation of the medulla. This center may be excited reflexly from the periphery, through the chemoreceptor trigger area or from rostral neural sites (*4*). It is adjacent to areas integrating and mediating vasomotor, respiratory, and postural responses and functionally related to adjacent and rostral brain-stem areas involved in extrapyramidal, visceral, and "alerting" functions. The emetic integrating mechanisms are thus linked with a complex of brain-stem operations that bring the individual physiologically into contact with both his internal and external environment. This complex of operations may mediate the change in level and quality of psychomotor and autonomic reactivity which characterizes tranquil behavior. The link between emetic mechanisms and tranquilization is thus a neural one.

Table 1. Proportion of dogs protected from emesis by dibenamine.*

Time to emetic challenge	Apomorphine dose (μg/kg)	
	2 × M.E.D. (60)	4 × M.E.D. (100)
20 min	0/4	0/4
2½ to 6 hr	3/3	3/6
24 hr	7/7	2/3

* In acid alcohol solution (0.4 percent), at a dose of 2 mg/kg intraperitoneally; apomorphine minimal emetic dose (M.E.D.) established at 25 μg/kg.

Apomorphine has central effects other than an emetic action. These apomorphine actions implicate the adjacent neural systems—for example, a hypotonic effect on spasticity produced either by decerebration or anterior cerebellar section (*5*) and, in the human, with subemetic doses, a tranquilizing action (*6*). It is entirely possible that drugs inhibiting the action of apomorphine at the chemotrigger area do so in part by action on these adjacent systems, which in turn may affect the reactivity of the emetic center.

One cannot use tests of emetic reactivity indiscriminately to infer tranquilizing or antiemetic clinical utility. Reserpine, for example, antagonizes apomorphine emesis in the dog but in the pigeon and in man may produce nausea and vomiting (*7*). Interpreting apomorphine tests, one may infer a brain-stem site of action; this is indicated by positive results and not at all ruled out by negative findings.

This report describes a centrally mediated antiapomorphine effect of dibenamine. A powerful adrenergic blocking agent, the drug acts peripherally on the effector cell to block chiefly the excitatory effects of epinephrine. Nickerson (*8*) has cited two phases in dibenamine activity: an initial epinephrine-dibenamine antagonism for the first 2 hours, and, with the binding of dibenamine to the peripheral effector cell, the onset of true adrenergic blockade enduring for 3 or 4 days. During the first phase, a brief period of central excitation may be noted, presumably affecting temporal lobe, as well as hypothalamic and medullary, function. Since a hydrolysis product of dibenamine which lacks adrenergic blocking properties produces the central effects of the first phase, the initial central effects may not be attributed to alteration of central adrenergic systems. Since no central effects had been experimentally demonstrable for the second phase, the finding of a prolonged tranquilizing action in anxiety states had to be ascribed to the peripheral blockade of adrenergic substances related to tension states (*9*), and the finding that catatonic patients were brought tranquilly into contact for a period of 18 to 72 hours was attributed to a possible action on central blood vessels (*10*). It would therefore be important to demonstrate an alteration of central neural activity that would be coincident with the behavioral effects and the peripheral adrenergic blockade of the second phase. Results of dibenamine inhibition of apomorphine emesis are indicated in Table 1.

With chlorpromazine and diphenhydramine, antiemetic potency is apparent during the peak period of pharmacologic activity (*1, 2*). With dibenamine, however, antiemetic potency is not demonstrable during the first 2 hours after administration. Following this initial period, and for a period up to 24 hours (the longest interval tested), a definite inhibition of apomorphine-induced emesis has been observed. The prolonged action thus establishes a central neural basis for the noted behavioral effects. This action is related neither to the adrenergic blockade (sympathectomy does not alter apomorphine emesis) nor to a clinical antiemetic effect. Both the excitatory phase and the local gastric irritation can produce nausea or vomiting.

The demonstration of prolonged central neuronal alteration does raise the possibility that less toxic agents with greater tranquilizing potency may be found among some of the interesting chemical analogs of dibenamine (*8*). From the experimental viewpoint, several clues point to particular receptor systems altered by dibenamine (*6, 8, 11*), so that the drug may now prove useful as a tool to study central drug-enzyme systems.

The fact that a drug may induce long-enduring central changes the neural bases for which are masked, stresses the importance in neuropharmacology of searching for such latent actions. In this respect, the apomorphine test of medullary vomiting mechanisms may be but one of several possibilities for studying brain-stem reactivity in attempting to develop drugs that affect psychic function.

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