MINIREVIEW

MIXED DEPRESSANT AND STIMULANT ACTIONS OF MORPHINE AND THEIR
RELATIONSHIP TO BRAIN ACETYLCHOLINE*

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Morphine and related narcotic agonists produce mixed depressant and/or stimulant effects in a wide variety of animals including man (1,2). These effects are not only species but also time and dose related. It is not generally appreciated that even a single dose of morphine in a given species, such as the rat, may have initial depressant and subsequent stimulant effects. We believe this is an extremely important concept and intend to provide evidence of its wide applicability in the pharmacology of narcotics.

It is well known that the principal pharmacological actions of narcotic agonists include effects on the central nervous, cardiovascular and gastrointestinal systems. For our purposes, we will stress narcotic pharmacology related only to the central nervous system. The interaction of morphine with the putative neurotransmitters reflects its complex pharmacology. There is now considerable evidence to implicate morphine and related narcotic agonists in acting on neurotransmitter systems, both centrally and peripherally (3-5). Inasmuch as acetylcholine (ACh), catecholamines (CA), and 5-hydroxytryptamine (5-HT) have all been implicated, it behooves investigators to define precisely the significance of the interaction of narcotic agonists with their favorite neurotransmitter. Our laboratory has been involved in studying the interactions of morphine with brain ACh. In this minireview we propose to analyze the role of ACh in some complex biphasic actions of morphine.

The major narcotic agonist hypothesis involving ACh is that it reduces ACh release from neural tissue, presumably directly from cholinergic neurons. This antirelease effect, however, is directly related to a number of factors that will be discussed below. Although narcotics inhibit in vitro and in vivo acetylcholinesterase and choline acetyltransferase (6), their major effects on cholinergic neurons can best be explained through their ACh antirelease action.

Methodological Considerations

Methodological considerations are important to discuss because of the complex nature of morphine pharmacology, problems in determining endpoints of CNS cholinergic effects, and in accurate ACh measurement. It is not our purpose to review morphine's pharmacology, but only to point out that when studying a drug with such wide ranging effects, one must consider the dosage and route of administration (7), duration of action and, most importantly, different effects during the time course. This latter point is exceedingly important in studying

* Supported in part by grant DA-00830 from the USPHS.
** Predoctoral Fellow, GM 00198-15, USPHS.
ACh and morphine because biphasic effects are noted (8,9). Too often, literature on morphine is not satisfactory because complete dose-effect studies are not done and/or the studies are not carried out for a long enough period of time.

Another factor to consider is the time of the day before morphine administration. In our laboratory, different effects were seen with morphine, depending on the circadian rhythm. Morphine at 32 mg/kg i.p. 30 minute pretreatment caused an increase in content of rat brain ACh 2 hours into the dark phase of the circadian rhythm but had no effect 2 hours into the light phase. In addition, control levels of ACh have been shown by many to be dependent on the circadian rhythm (10-12).

Changes in dosage, time, duration and pre-experimental conditions are not the only important considerations when dealing with morphine pharmacology. One must also consider species variation. There is a primary excitatory component with varying doses of morphine in mice and cats. Other species, such as the rat and monkey, exhibit depressant effects at equivalent doses. Furthermore, the rat shows biphasic pharmacological effects not only with dosage but also with time after a single dose. For the following parameters: body temperature, motor activity and EOG (13-24). Morphine in a dose of 20 mg/kg i.p. produces an initial depression of rat locomotor activity for about 1-2 hours, depending upon the route of administration, followed by another 2 hour period of hyperactivity (25). We have duplicated this finding using doses of 1.0, 3.2, 10 and 32 mg/kg of morphine sulfate, dose calculated as base, as illustrated in Fig. 1. The initial depressant or cataleptoid effect lasts longer with larger doses. It is possible that morphine may have both depressant and excitatory effects in all species and that the reason depression or stimulation is not observed may be due to variations in dose and time after administration. It is important, therefore, when interpreting data on morphine to take into account all of the above considerations. This is especially important when studying the effects of morphine on brain neurotransmitters.

Particularly in the case of brain ACh, other methodological problems to consider are the technique of sacrifice, assay of ACh, and the best parameters for measuring cholinergic function, i.e., total or regional brain levels, utilization, or turnover.

It has long been a problem when working with the cholinergic system to have at one’s disposal accurate and sensitive methods of assaying ACh. Quite recently, however, a number of new techniques have been developed for sensitive assays (26). These techniques include gas-liquid chromatography (27), colorimetry and fluorometry (28-30), and various radiochemical methods (31-34) which have replaced bioassay procedures.

In our laboratory several ACh assay techniques have been used over the past few years. The frog rectus (35) and leech muscle (36) assays have been replaced by a modification of the pyrolysis gas liquid chromatographic assays (27,37) as described elsewhere (38,39). Although bioassay procedures have a high sensitivity, there were many inherent problems, including the variable response of the preparation, possible interaction with other agonists, etc. The pyrolysis-GLC technique, however, has some inherent problems as well. The number of samples that can be run at one time is limited and the sensitivity of this method is poor in our hands.

Another problem involved with measuring brain ACh, particularly total or regional levels, is the method of sacrificing the animal. We have tried various methods of sacrifice to obtain the maximum values of rat brain ACh on the assumption that these represent the in vivo condition. The data obtained is
Biphasic effects of morphine on locomotor activity in 28 day old rats

Male albino Holtzman rats were given s.c. morphine sulfate in the above doses calculated as base. Mean activity counts/hr were obtained in a 4 photocell activity chamber. Animals were run for 7 hr beginning 1 hr into their light cycle. The N for each group is 6-8. An iso-osmolar sodium sulfate control was used. Symbols in this and subsequent figures using a group comparison "t" test are: *P < .05, **P < .02, ***P < .01, ****P < .001.

In determining functional changes of any neurotransmitter, one can measure brain content, either total or regional, turnover, or release. Total brain ACh is a very limited indicator that a drug may be affecting the central cholinergic system. Steady state levels do not necessarily reflect changes in ACh metabolism.
or drug effects in discrete neuroanatomical areas.

The study of turnover has provided an even more meaningful method to study drug effects. Changes in ACh metabolism can directly reflect a drug's action. Turnover can be described as the process of renewal of a substance in a tissue by synthesis in a given compartment, or elsewhere and transported into the compartment (41). We have divided turnover methods into direct and indirect. Direct turnover studies involve the use of drugs or other means to immediately stop ACh synthesis or degradation, as well as the use of a labelled precursor. With direct methods it is assumed that the transmitter enters by synthesis, leaves by degradation, does not diffuse out of the brain and that turnover remains essentially constant. Another approach is the indirect measurement of brain ACh turnover. Compounds that inhibit ACh synthesis (but do not have immediate effects) are given intraventricularly (ivt.). One such class of compounds, the hemicholiniums, does not penetrate the blood brain barrier readily so their actions are limited primarily to the CNS. With synthesis of ACh reduced, drugs can be given at various times in order to determine if brain levels of ACh are changed. A drug that lowers ACh below or above the level found with the ACh depletor alone causes an increased or decreased utilization of ACh, respectively. The term "utilization" was employed since this indirect method does not meet the criteria for direct turnover. We have used HC-3 and acetylseco HC-3 to inhibit the synthesis of ACh. Acetylseco HC-3 is used preferentially because of its dual mechanisms of action (inhibition of choline uptake and choline acetyltransferase), it provides less variability, and it is less toxic (42). Procedures using these compounds are detailed elsewhere (35,43).

The release of ACh from the CNS has provided another means to study the effects of narcotics. Studies have been done with cortical slices, intact cerebrum with perfusion of cerebral ventricles and cortex, and via chronically implanted cups. All of the experiments conducted in our laboratory were done with cortical cups in unanesthetised pretrigeminal transected rats (44) and cats (45). In these brainstem transected animals morphine caused a decrease in ACh release from the cortex. Many other investigators (46-48), in addition to

### TABLE 1

Methods of Sacrifice and Extraction for Optimal Amounts of Rat Brain ACh

<table>
<thead>
<tr>
<th>Means of Death</th>
<th>Extraction and Assay Method</th>
<th>N</th>
<th>Mean ± S.E. ACh/g brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guillotined</td>
<td>Acid alcohol, frog rectus</td>
<td>9</td>
<td>9.5 ± 0.5</td>
</tr>
<tr>
<td>Brain frozen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frozen alive</td>
<td>Acid alcohol, frog rectus</td>
<td>11</td>
<td>15.8 ± 0.8</td>
</tr>
<tr>
<td>Liquid N₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frozen alive</td>
<td>Acid alcohol, frog rectus</td>
<td>8</td>
<td>15.3 ± 0.8</td>
</tr>
<tr>
<td>Freon-12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guillotined</td>
<td>Acid alcohol, pyrolysis-gas chromatography</td>
<td>9</td>
<td>18.7 ± 0.4</td>
</tr>
<tr>
<td>Microwaved</td>
<td>Acid alcohol, pyrolysis-gas chromatography</td>
<td>17</td>
<td>20.5 ± 1.0</td>
</tr>
<tr>
<td>Guillotined</td>
<td>Acetonitrile, pyrolysis-gas chromatography</td>
<td>42</td>
<td>25.8 ± 0.4</td>
</tr>
</tbody>
</table>
ourselves, have found that morphine in the lightly anesthetized animals caused a decreased release of cortical ACh. This is also seen in unanesthetized, freely moving rabbits (49). However, similar doses of morphine cause ACh release from the cortex of unanesthetized cats (50). It appears, therefore, that further work in this area should involve unanesthetized, freely moving animals with chronic implanted cups or cannulae. Anesthetized animals should not be used for many anesthetics reduce ACh release from the neocortex (51). In addition, further dose-time studies should be done to detect a morphine depressant-stimulant action on ACh release as discussed below.

Biphasic Effects of Morphine on Various Behaviors

A. Locomotor Activity

It is well documented that morphine produces a cataleptoid depression in naive rats in doses greater than 10 mg/kg (52,53). Small doses (1.0 to 4.0 mg/kg) produce an initial excitation (25,54). Although many investigators have measured motor activity after morphine (21,55-57), Babbini and Davis stressed the dose and duration aspects. They found that morphine in doses of 1.25, 2.5, and 5 mg/kg i.p. had initial stimulant effects with no depression. Doses of 10, 20 and 40 mg/kg i.p. caused biphasic effects. As mentioned above, we replicated this important observation using a smaller dose range, as illustrated in Fig. 1. As the dose of morphine increases, the depression of locomotor activity lasts for a much longer period of time. Note that 1.0 mg/kg s.c. showed no depression, only stimulation, and that 3.2 mg/kg s.c. showed only slight, transient locomotor depression. In mice apparently no biphasic effects are seen as one varies morphine dosage. Doses of 30, 100 and 300 mg/kg of morphine i.p. all caused a dose related increase in locomotor activity (58). The data of Kuschinsky (59) confirm those of Rethy et al. that doses of 10 mg/kg and above increase activity while 5 mg/kg did not. No sedative component was seen with any dose of morphine given to mice. However, CD-1 mice show initial depression (60).

In the cat, initial sedative and subsequent excitatory effects of morphine have been noted. This consists of an initial period of depression, an intermediate period of reorganization, and a third period of ritualization, which consists of stereotyped locomotor patterns (61). The last two periods are the major components of the well known stimulatory phase of morphine in the cat. The effects of morphine on motor activity demonstrate the complexity of the responses seen with this drug. If one were to look at only one dose of the drug at different time periods, the conclusions drawn would be quite different from those formulated by doing systematic dose-effect and time-effect studies.

B. Self-Stimulation

Morphine has been shown to have mixed depressant and stimulant effects on operant behavioral tasks (62). Studies with morphine on intracranial self-stimulation have been especially of interest. Both are very potent positive reinforcers. Olds and Travis (63) showed that morphine depressed rat self-stimulation behavior. Recently it has been shown (64) that morphine in doses of 5, 10, and 20 mg/kg s.c. showed biphasic effects in self-stimulated rats (Fig. 2). Up to 2 hours after morphine, depression of responding was observed. Maximum responding was seen at 3-4 hours with 10 mg/kg and at 5 hours with 20 mg/kg. Their 10 mg/kg time effect curve corresponds well with our 10 mg/kg data on locomotor activity (compare Figs. 1 and 2). It is apparent that morphine exhibits biphasic effects in the rat, as demonstrated by these two different behaviors. The correlation with time between the two would suggest that these phenomena may be related and have similar neurotransmitter mechanisms.
Biphasic effects of morphine on hypothalamic self-stimulation in the rat

Mean number of lever presses per 10 min for rewarding hypothalamic stimulation during the control (C) period and for each hr after the s.c. injection of morphine or 0.9% NaCl (saline). Data reprinted from Lorens and Mitchell (64) with permission.

Biphasic Effects of Morphine on Various Physiological Endpoints

A. EEG Effects

Khazan and his associates (14,15) have shown that morphine has two major effects on the sleep-awake cycle of the rat. The drug initially produces stupor followed by arousal. In doses of 10 mg/kg of morphine sulfate i.p., the duration of each phase is about 90 minutes, as illustrated in Fig. 3. However, another report (65) stresses 5 phases of EEG slow waves after a transient initial EEG desynchrony following 40 mg/kg of morphine sulfate s.c. Whether EEG slow waves are manifestations of "depression" or "excitation" also is interpreted differently by these investigators. Gunne (17) has emphasized that in the morphinized rat both gross depression and excitation are observed. There are 2-5 hours of somnolence, depending on dose, followed by 1-2 hours of increased activity.

B. Body Temperature

An action of morphine which is thought to have a subcortical locus, probably the hypothalamus, is temperature regulation. Chahovitch and Vichnjitch (66) showed that low doses of morphine caused hyperthermia in the female rat. Later, Herrmann (18) confirmed this and also determined that morphine in doses above 30 mg/kg caused an initial hypothermia. As reproduced in Fig. 4, the data of Gunne (17) indicate that morphine in various doses (10-60 mg/kg s.c.) in
The awake-sleep cycle is plotted graphically over time under control 0.9% NaCl, morphine and morphine-naloxone treatments. Note that 10 mg/kg of morphine sulfate i.p. to a female Sprague-Dawley rat has two actions. The first is to produce stupor and EEG slow waves which last about 90 min. The second produces activation which also lasts about the same period of time. The narcotic antagonist, naloxone, prevents the action of morphine. Reproduced from Colasanti and Kharaz (14) with permission.

female rats has a complex biphasic effect. Lower doses show a hyperthermia, while higher doses show an initial hypothermia and a subsequent hyperthermia. These effects should be compared with the initial depressant and subsequent excitatory effects of morphine on locomotor activity or self-stimulation. It is important to point out, however, that the time course of the effects on body temperature does not correlate completely with the behavioral data. We are currently undertaking a study in male rats in an attempt to correlate these different effects of morphine.

Morphine Antinociceptive Effects

The intensity and duration of morphine analgesia in the rat depends upon the dose and route of administration. Its antinociceptive effects usually last
Effect of morphine on mean body temperature in the rat

The mean rectal temperature of groups of 8-14 female albino rats is given for various doses of morphine hydrochloride s.c. The broken lines represent the rectal temperature of 40 0.9% NaCl controls and the solid lines represent the rectal temperature after morphine. In A, a pure hyperthermic response was observed to 10, 20 and 30 mg/kg of morphine and in B a complex initial hypothermic and subsequent hyperthermic response to 40-60 mg/kg of morphine. The temperature effects of morphine are not only dose-dependent, but are relatively long lasting for 3-6 hrs depending upon dose. Reproduced from Gunne, L.-M. (17) with permission.

for several hours using heat (7), tail pinch (6), or a flinch-jump procedure to electric footshock (67). The time course of the morphine sulfate antinociceptive effect as a function of total dose given i.p. is shown in Fig. 5. It can be noted that 3 mg/kg of morphine causes analgesia for about 90 minutes, while 6 and especially 9 mg/kg act for several hours. There is appreciable analgesia at 90 min with the larger doses of morphine. One's first impression is that analgesia, at least in the rat, seems to correlate with the initial actions of morphine. Furthermore, the decrease in ACh release from the neocortex also seems to correlate with the initial depressant effects of morphine. Morphine analgesia, however, outlasts the reduction in ACh release which in the pretrigeminal brainstem transected rat lasts only about 90 minutes in doses of 10 mg/kg (Fig. 6). In the cat, apparently there is a marked discrepancy between the duration of ACh antirelease from the cortex and morphine analgesia. In acute pretrigeminal midpontine transected cats 1.0 mg/kg of morphine i.v. reduced cortical ACh release for about 90 minutes (45). The time course of morphine sulfate (1 mg/kg, i.v.) in elevating tooth pulp thresholds in the chronic cat according to Mitchell (68) lasts for several hours. At 90 and even 120 minutes after morphine, there is still a considerable increase in the tooth pulp threshold.

Effects of Morphine on Brain ACh

A. Total Brain Content

As described above, many studies have been performed on the effect of narcotic agonists on total brain ACh. As shown in Table 2, the route and time
after administration of morphine caused varying changes in brain ACh of mice and rats. The collective data indicate that the increase of brain ACh occurs at doses higher than those required for analgesia. It should be noted that the % increase varies considerably. Thus, one must consider route, time and species for the peak effect in brain ACh. Very few investigators have done systematic dose and time effect curves.

B. Brain ACh Turnover

As described earlier, changes in total brain ACh do not give any specific information on changes of discrete areas and metabolism of cholinergic neurons in the central nervous system. Turnover measurements can provide better correlations with drug action and a putative transmitter. Direct turnover studies of the effects of morphine on brain ACh have been made (79,80). Morphine causes a decrease in total brain ACh turnover in the mouse and a decreased ACh turnover in the rat cortex but not in the rat striatum, suggesting important regional brain differences and the necessity of examining many different brain areas.

As described above, for practical reasons we have used an indirect measure of total rat brain ACh turnover using HC-3 and acetylsec HC-3 given ivt. in doses which would reduce brain ACh and give a reasonable percentage of survivors.

A survey of various prototype compounds from the morphine, morphinan, ben-
Groups of 5 female albino Sprague-Dawley rats (per point) were given i.p. morphine sulfate. Total dose is given in parentheses. On the "y" axis is plotted the % increase in control threshold to electric footshock using a flinch-jump endpoint. Reproduced from Tilson et al. (67) with permission.

Zomorphan, phenylpiperidine, and diphenylmethane series of narcotic analgesics were studied. As shown in Fig. 7, all of the agonists from these different chemical classes had anti-ACh depleting actions at the designated time. Furthermore, the quantitative differences between the various classes of agonists were related to their known potency as analgesics. Heroin was more potent than levorphanol > di-methadone > phenazocine > morphine > codeine > meperidine. It should be noted that the data obtained for heroin was obtained using a modified ACh assay of Schmidt et al. (37), while that for the rest of the narcotics was with the frog rectus bioassay. This is probably a valid comparison because the dose-effect data on morphine against HC-3 are similar using the two different assay methods (38). However, what is strange and bears reexamination is that heroin is surprisingly more potent than morphine (by 10 times) whereas it is only 3 times as potent an analgesic in man. Furthermore, the dose-effect relationships of heroin, morphine, and codeine differed. The most "stimulant" narcotic agonist, codeine, had an inverted "U" shaped dose response curve while morphine in the doses tested did not. We observed similar trends with 3.2 mg/kg of heroin. Larger doses of heroin were 100% lethal with HC-3. It should be emphasized that large doses of all of the narcotic agonists showed enhanced lethality when given with the HC-3. Because of the lethal effects, analysis of the high dose phenomenon was not practical. It would be of interest to know if rats in whom this combination was lethal had lower brain ACh levels than those who survived for 30 minutes. The weak narcotic agonist, propoxyphene, in a dose of 17.5 mg/kg i.p. caused a slight antagonism of HC-3 depletion of rat brain ACh (35).

Another approach we have taken is to look at the effects of morphine on
Time course of morphine reduction of acetylcholine release from the neocortex and antagonism by naloxone.

Note that morphine sulfate, in a dose of 10 mg/kg as base, i.p. reduces ACh release for about 90 min. This effect is antagonized by naloxone, 1.0 mg/kg, i.p. Each point represents the mean of 4-8 assays in two different groups of animals. Reproduced from Matthews et al. (44) with permission.

utilization after another ACh synthesis blocker, acetylseco HC-3. The results are shown in Fig. 8. Morphine (10 mg/kg or 32 mg/kg s.c.) was administered at various pretreatment times. Thirty minutes prior to sacrifice 0.32 or 5 μg of acetylseco HC-3 were injected ivt. Animals were sacrificed by guillotine and ACh extracted and assayed as described previously (39). At 30 minutes 10 mg/kg of morphine caused a highly significant increase in ACh content. At one hour the higher dose also caused a dramatic increase in content, indicating decreased utilization. At 3 hours, however, 10 mg/kg of morphine caused a significant depletion in ACh, implying an increase in its turnover. This data would be in complete agreement with the initial depressant, subsequent stimulant actions of morphine. The decreased then increased utilization correlates with the initial depressant, then stimulant actions of morphine on the behavioral parameters mentioned above.

C. Cortical Antirelease-Release of ACh

As described above, many investigators found that morphine causes a decreased release of brain ACh. In the rat to date we have only studied morphine and nalorphine as ACh antirelease agents (44). As mentioned above, a dose of 10 mg/kg of morphine i.p. reduced ACh release in the pretrigeminal brainstem transected rat for only about 90 minutes (Fig. 6). The mixed antagonist-agonist
Antagonistic Effect of Various Narcotic Agonists on HC-3 Depletion of Brain Acetylcholine

The ordinate indicates the change in ACh from rats given 20 μg of HC-3 to those given 20 μg of HC-3 and a narcotic agonist. The dose given i.p. is plotted on the abscissa. As the dose of the agonist increased, there was a corresponding increase in the amount of brain ACh present. The agonists fall in line with their analgesic potency in man except for heroin which seems more potent in this test.

Biphasic Effects of Morphine on Brain ACh Utilization in the Rat

Animals were pretreated with 0.32 or 5 μg acetylseco HC-3 (ivt.) 30 min prior to sacrifice. Morphine sulfate (as base content) was injected s.c. at the times indicated. Each column represents 8 or more animals.
nalorphine (1.0 and 10 mg/kg) had a similar but much shorter antirelease effect while the pure antagonist naloxone (10 mg/kg) had no significant effect.

In the pretrigeminal brainstem transected cat the same morphine derivatives were studied with similar results. In the cat, nalorphine in a dose of 1.0 mg/kg i.v. seems to have a longer ACh antirelease action than a similar dose of morphine. Again, the pure antagonist naloxone, 1.0 mg/kg i.v., had no effect. As might be expected, levorphanol was more potent than morphine in reducing ACh release. However, the dose effect relationships of both narcotic agonists were unexpectedly biphasic. Within the first hour, small doses of both agonists produced ACh antirelease with a maximal depression of about 50% in 30 minutes. However, large doses of both compounds enhanced ACh release in the cat. It should be noted that such doses produce marked behavioral excitation in this species. Furthermore, i.p. or i.v. administration of certain doses of morphine to unanesthetized cats caused a dose related increased release into the cerebral lateral ventricle and cerebral cortex (81). A one hour localized perfusion of the cat sensorimotor cortex with a 1-5% solution of morphine had no effect on ACh release (82). This would indicate that the narcotic must act subcortically to reduce ACh cortical release.

Conclusions

1. It is apparent that in most species of animals morphine, even in small doses, has mixed depressant and stimulant actions. The critical question of whether narcotic euphoria and drug seeking behavior are due to its depressant or stimulant effects is still unanswered.

2. The initial behavioral depressant actions of morphine seem to be correlated with a decrease in brain ACh utilization and/or turnover, and the subsequent stimulant actions with an increase in ACh utilization and/or turnover.

3. It is important that future studies take into account the various parameters (dose, time, species, etc.) discussed above in drawing conclusions about the complex pharmacological effects of morphine.

References


68. C. Mitchell, Unpublished observations (1972).


