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Brain Acetylcholine in Morphine Pellet Implanted Rats Given Naloxone *

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Abstract. Adult male rats were implanted with intraventricular (ivt.) brain cannulae for injection of 5 µg of acetylseco-hemicholinium-3 (acetylseco HC-3) as a means of studying acetylcholine (ACh) utilization during morphine withdrawal. Animals were made dependent by implanting s.c. two 75 mg morphine base pellets 24 hrs apart. On the 4th day animals were given 10 mg/kg of naloxone i.p. and/or 5 µg acetylseco HC-3 ivt. and sacrificed by decapitation at various times. The brains were removed and assayed for ACh using a pyrolysis gas chromatographic procedure. Total brain ACh before or after acetylseco-HC-3 was not altered at 5, 30, 60 and 120 but was decreased at 10 min after naloxone. These results are in sharp contrast to our previous data of enhanced brain ACh utilization in withdrawn rats made dependent to morphine by several weeks of twice daily injections. It is apparent that short term morphine pellet administration does not produce the marked neurochemical and behavioral changes of long term morphine injections.

Key words: Acetylcholine – Acetylseco-Hemicholinium – Morphine – Naloxone.

The pellet method of studying morphine tolerance and withdrawal is now widely used because of rapidity of tolerance development and obvious convenience to investigators (Way *et al.*, 1969; Wei, 1972). Recently, Cheney and Hanin (1973) reported that acute morphine administration reduced mouse brain acetylcholine (ACh) turnover. They were able to show rapid tolerance development to this phenomenon in morphine pellet implanted animals. Their elegant studies of brain ACh turnover confirmed our previous findings (Domino and Wilson, 1973a) in the rat using indirect means of measuring ACh utilization via intraventricular (ivt.) hemicholinium-3 (HC-3) induced ACh depletion and its antagonism by morphine and other narcotic agonists. We also reported that during morphine withdrawal in dependent rats previously given the drug bid for 8–12 weeks there was increased utilization and a decrease in total brain ACh (Domino and Wilson, 1973b). Using a different approach Large and Milton (1970) came to a similar conclusion. Recently, Bhargava and Way (1974) showed that brain ACh is reduced in morphine pellet treated mice and rats given naloxone, and that the ACh decrease was associated with increased jumping during withdrawal. Hence, it was quite surprising that Cheney and Hanin were unable to show any change in brain ACh turnover in morphine pellet implanted mice 15 min after naloxone injection. We argued that one must study the complete time course of narcotic withdrawal for a biphasic effect on ACh release is known to occur (Labrecque and Domino, 1973). Thus, 15 min may be the critical inflection point for no change in ACh turnover. Nevertheless, the Cheney and Hanin study was sufficiently disturbing to cause us to reexamine the problem in morphine pellet rats using those techniques of ACh utilization with which we are familiar.

The present manuscript describes findings in the rat which are similar to those obtained by Cheney and Hanin, as well as Bhargava and Way in the mouse and rat. However, our data raises serious questions regarding the significance of short term morphine pellet implantation as a means of studying morphine dependence.

Methods

Male Holtzman rats 250–300 g were housed 6 to 10 in group cages under previously described conditions (Domino and Wilson, 1973a, b). Rats were used one week after implantation with ivt. polyethylene cannulas by the method of Robinson *et al.* (1969) and Altaffer *et al.* (1970). Rats receiving

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morphine (75 mg base) pellets were implanted 3 days prior to use by the method of Wei (1972). Two pellets were implanted 24 hrs apart. In one series the pellets were not removed and during another they were removed 6 hrs prior to withdrawal. Withdrawal was precipitated by 10 mg/kg of naloxone hydrochloride i.p. calculated as base. Acetylseco hemicholinium-3 (acetylseco HC-3) was given to unanesthetized slightly restrained rats via the ivt. cannula $^1/_2$ hr prior to sacrifice. All animals were guillotined at approximately the same time (8:00–9:00 a.m.) each day. Rat brain ACh was determined by gas liquid chromatography per the methods of Schmidt *et al.* (1972) and Szilagyi *et al.* (1972). After guillotine, brains were removed and homogenized in acetonitrile with 25 nmol of proprionylcholine as internal standard. A Hewlett Packard 5750 gas chromatograph with 8 ft. by $^1/_4$ in. stainless steel columns packed with 20% Carbowax 6000 on 60/80 mesh Chromasorb W (HMDS) was used for analysis.

Results

In Table 1 are given mean brain ACh ± S.E. in nmol/g wet weight after various procedures. It should be noted that mean brain ACh ± S.E. of control animals was 18.1 ± 1.3 with the Schmidt *et al.* procedure and 24.6 ± 0.7 with the Szilagyi *et al.* procedure. Rats which received no treatment of any kind did not significantly differ in brain ACh from those that were cannulated, contained morphine pellets for 72 hrs or sham pellet rats that received 10 mg/kg naloxone $^1/_2$ hr prior to sacrifice. The ivt. injection of 5μg of acetylseco HC-3 to control animals caused a significant decrease in brain ACh to 10.1 ± 0.6 nmol/g. This dose of acetylseco HC-3 was chosen because it gave a moderate depletion of rat brain ACh which could be used as an indirect indicator of ACh utilization (Domino and Wilson, 1973 a, b). Mean brain ACh ± S.E. at various times of withdrawal with and without 5 μg ivt. acetylseco HC-3 are also given in Table 1. When 72 hrs morphine pellet rats received 10 mg/kg naloxone there was no significant change in brain ACh at $^1/_2$ hr whether the pellet was present or had been removed 6 hrs previously. During the first 4 min after naloxone animals exhibited exploratory behavior, wet shakes, blanching of ears and tachypnea. A marked sedation was observed 5–8 min later with diarrhea, vocalization on touch, rhinorrhea and ptosis. Morphine pellet rats given 5 μg ivt. acetylseco HC-3 in addition to naloxone exhibited similar signs. Brain ACh was decreased but not significantly above or below levels of brain ACh after 5 μg of ivt. acetylseco HC-3 given alone to control animals. Only 10 min after naloxone was there a significant decrease in brain ACh in the morphine pellet animals.

Table 1. Mean brain ACh ± S.E. nmol/g

Treatment	Alone			Plus 5 μg ivt. acetylseco HC-3		
	N		A *p* value	*N*		B *p* value
Schmidt et al., ACh assay						
Controls	13	18.1 ± 1.3	NS	–	–	NS
Morphine pellet (72 hrs)	8	18.4 ± 0.7	NS	–	–	NS
Cannulated rats	8	18.2 ± 0.4	NS	9	10.1 ± 0.6	<0.001
Sham pellet, 10 mg/kg naloxone	8	18.6 ± 0.3	NS	7	10.1 ± 0.3	<0.001
Sham pellet, 10 mg/kg naloxone pellet removed	8	17.7 ± 0.9	NS			
$^1/_2$ hr withdrawal, 10 mg/kg naloxone pellet not removed	17	18.9 ± 2.4	NS	9	11.2 ± 0.5	<0.001
$^1/_2$ hr withdrawal, 10 mg/kg naloxone pellet removed	8	16.4 ± 0.5	NS			
1 hr withdrawal, 10 mg/kg naloxone pellet not removed	8	19.6 ± 0.7	NS	8	10.1 ± 0.4	<0.001
2 hrs withdrawal, 10 mg/kg naloxone pellet not removed	8	18.6 ± 1.1	NS	7	11.0 ± 0.4	<0.001
Szilagyi et al., ACh assay						
Controls	7	24.6 ± 0.7	NS	14	14.2 ± 0.5	NS
5 min withdrawal, 10 mg/kg naloxone pellet not removed	8	23.5 ± 0.6	NS	7	13.1 ± 0.6	NS
10 min withdrawal, 10 mg/kg naloxone pellet not removed	8	21.6 ± 0.7	<0.05	9	14.0 ± 0.6	NS

A Group comparison Student "*t*" tests of appropriate control vs withdrawn rats.
B Group comparison Student "*t*" tests of alone vs rats with 5 μg ivt. acetylseco HC-3.

Discussion

The rat brain ACh levels of pellet animals during morphine withdrawal differ considerably with those we found in withdrawing rats given weeks of morphine by injection (Domino and Wilson, 1973 a, b). Instead of a decrease, there was no change in the morphine pellet present or the morphine pellet absent animals 5, 30, 60 and 120 min after naloxone. Only 10 min after naloxone were total brain ACh levels reduced in the morphine pellet animals ($P<0.05$). There was no significant change in brain ACh utilization as measured indirectly by acetylseco HC-3. The morphine pellet present or absent rats given naloxone showed obvious withdrawal since their signs corresponded to those described by Wei (1972). Rats did not show any withdrawal symptoms 6 hrs after pellet removal. The major deficiency of this study was our failure to quantify the degree of morphine withdrawal the animals were experiencing. This is especially critical in view of the observation by Bhargava and Way (1974) that only rodents which jumped during morphine withdrawal showed decreased brain ACh levels.

Naturally withdrawn rats after weeks of a narcotic agonist show an increase in motor activity (Akera and Brody, 1969; Martin *et al.*, 1963). On the other hand, rats tolerant to morphine via injection or pellet implantation given a narcotic antagonist show immobility (Kaymakcalan and Woods, 1956; Large, 1972; Wei, 1972) with return to normal activity. Rats undergoing withdrawal after weeks of a narcotic agonist experience prolonged stress. In our morphine pellet animals at the end of 2 hrs of withdrawal there was a 6–8% weight loss. In our naturally withdrawn chronically treated rats there was a 14–20% weight loss after 48 hrs. Akera and Brody (1969) have noted that at 48 hrs withdrawal symptoms were most prominent in such chronic morphine dependent animals. Tilson *et al.* (1973) have also described the greatest decrease in nociceptive threshold at this time.

It should be noted that Colasanti *et al.* (1974) found that 72 hrs morphine pellet implanted rats upon removal of the pellets show much shorter rebound REM sleep time during abstinence than after prolonged i.v. morphine (Khazan and Colasanti, 1972). These investigators concluded that their i.v. method produces a greater degree of drug dependence. Recently, Bläsig *et al.* (1973) have studied the development of physical dependence on morphine in rats implanted s.c. with morphine containing pellets in which the dosage, frequency of implantation, and duration of exposure to morphine were varied. These investigators showed that the various signs of precipitated withdrawal do not all increase concomitantly with increasing dependence. It is therefore quite possible that observed changes in ACh turnover are associated with the manifestation of a particular behavior rather than an underlying physiological mechanism relevant to physical dependence *per se*.

It appears that morphine withdrawal after pellet implantation, while producing many of the physical signs of withdrawal, does not affect brain ACh similar to rats receiving morphine over many weeks. The symptoms are on entirely different time scales and much more compressed in the pellet withdrawing rats. In addition, dependent rats given morphine by injection show excitation immediately after the morphine. Such rats subjected to prolonged exposure to morphine, whether withdrawal is by an antagonist or naturally, have different brain levels of ACh than pellet withdrawing animals given an antagonist. Large (1972) found that nalorphine precipitated abstinence in tolerant rats may be manifested by either excitement or depression, depending on frequency of injection, the strain of the rats, whether they received a regular dose of morphine before the antagonist, or when the antagonist was used in place of the morphine. Chronic exposure to a narcotic agonist undoubtedly causes biochemical and physiological changes that do not have time to occur in the 3 day morphine pellet rats. Thus, the method used to produce physical dependence to narcotics and the manner in which withdrawal is precipitated should be carefully considered. The changes that occur in brain ACh content and turnover are on different time scales, intensities, and can be in opposite directions. Furthermore, it is not valid to make comparisons between two modes of drug administration when drug dosages, brain levels, and time course are not comparable. Obviously such studies need to be done. The present results merely emphasize the need for more careful investigations and caution the indiscriminant use of morphine pellets without such quantitative data.

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