transducers of 20 MHz were mounted on these surfaces to generate shear vibrations in the (100) direction. The conventional "pulse transit" technique[8] was used to determine the transit time of ultrasonic pulses through the specimen. The procedure for calculating velocity and C_{44} from the measured transit time is elementary; Murnaghan's equation of state, together with the elastic constants determined by Bartels and Schuele[9], were used to estimate sample density and the change of its dimension under pressure. Accuracy in the velocities is estimated to be 1%; relative precision among the measurements is better than 0.1%.

Both the velocities and the corresponding values of C_{44} are plotted against pressure in Fig. 1. The pressure derivative of C_{44} at 1 atm is found to be -0.31, in good agreement with the determination of Bartels and Schuele[9]. A slight downward curvature appears in the C_{44}/P relation, but this curvature does not become great enough to bring C_{44} near to zero. The relatively large uncertainty in pressure determinations above 4 kbar does not allow an accurate determination of the magnitude of this curvature. I conclude that the phase transformation of KCl from its NaCl to the CsCl structure is not associated with the vanishing of C_{44}.

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BIOLOGICAL SCIENCES

Enhanced Utilization of Brain Acetylcholine during Morphine Withdrawal in the Rat

MORPHINE prevents the release of acetylcholine (ACh) from cholinergic neurones[1–9] when the neurones are stimulated at a low but not at a high frequency. Thus a special mechanism for neurotransmitter release at low frequencies seems to be involved[10]. Although the relationship of this effect of morphine to analgesia is unclear, it may be important to the sedative actions of morphine as well as to the phenomenon of physical dependence. Some aspects of the morphine withdrawal syndrome have a cholinergic component[11–13]. Morphine reduces the release of brain ACh[1–9,14,15] so it is to be expected that its turnover is reduced in acutely narcotized animals, and enhanced during withdrawal of chronic morphine dependent animals.

Direct measurements of ACh turnover have not been made in morphine dependent animals because of methodological complexities. Large and Milton[16,17], however, using the cholinesterase inhibitor, physostigmine, provided indirect evidence for enhanced ACh turnover during morphine withdrawal in the rat. This report describes data which are compatible with their conclusion, but which were obtained using two different inhibitors of ACh synthesis. One of these, hemicholinium-3 bromide (HC-3), presumably acts by preventing choline transport across various cellular membranes[18–21]. The other, acetylseco hemicholinium-3 bromide (acetylseco HC-3), is an inhibitor of choline acetyltransferase[22]. Intraventricular (i.v.t.) injection of these compounds reduces total brain ACh. This effect is reversed by morphine and related narcotic agonists and narcotic antagonists[23–25]. The technique is not a direct measure of brain ACh turnover. It is an acceptable, indirect approach to the study of ACh utilization because (a) the expected decrease in brain ACh was observed, (b) the incorporation of ^{14}C-choline into ^{14}C-ACh was clearly reduced (Domino *et al.*, unpublished results), and (c) data obtained with pentobarbital using this method are in complete agreement with those reported for pentobarbital using incorporation of ^{14}C-choline into ACh as a measure of turnover[26,27].

Male Holtzman rats (20–30 days old) were housed in group cages in an artificially illuminated room maintained at 24 ± 2° C. All animals were kept on an automatic 7.30 a.m. to 12.00 p.m. light and 12.00 p.m. to 7.30 a.m. dark cycle. Food and water were freely available. Morphine was administered twice daily at approximately 8.00 a.m. and 8.00 p.m. on the following schedule for the 2 week series: day 1, 10; day 2, 20; day 3, 30; day 4, 50; day 5, 60; day 6, 80; day 7, 100; day 8, 110; day 9, 120; day 10, 130; day 11, 150; day 12, 160; day 13, 180 and day 14, 200 mg kg^{-1}.

Animals given morphine for 8 weeks were on the following twice daily schedule: day 1, 20; day 2, 25; day 3, 30; day 4, 35; day 5, 45; day 6, 50; day 7, 55; day 8, 60; day 9, 65; day 10, 70; day 11, 75; day 12, 80; day 13, 85; day 14, 90; day 15, 95; day 16, 100, and 100 mg kg^{-1} for the remaining 6 weeks. Groups of at least twelve animals were run initially. There were six or more surviving animals per group. All injections were given intraperitoneally (i.p.) except in the animals receiving morphine for 8 weeks, where occasional subcutaneous (s.c.) injections were given to reduce mortality. Control animals were given 0.9% NaCl. After chronic administration of morphine for 15 or 57 days, animals were given, in the morning at the regularly scheduled time, a narcotic or saline, i.p., and HC-3 and/or acetylseco HC-3, i.v.t., and 30 min later killed by decapitation. All animals were given diethyl ether–air anaesthesia for the i.v.t. injections and allowed to recover. Animals were killed after 30 min because the rate of depletion of ACh was relatively linear at this time. ACh depletion was almost maximal with a survival rate of approximately 80%[23–25]. Total ACh was extracted from brain tissue (minus cerebellum) with the acid alcohol method of Stone[28] and bioassayed using the frog rectus abdominis method as described by Feldberg[29]. Hydrolysed tissue extracts to which ACh standard was added were used to account for the presence of sensitizing factors. All drugs were given in conveniently available salts and dosage calculated as base content. Because HC-3 and acetylseco HC-3

Table 1 Effects of Various Control Procedures, Morphine and Nalorphine on Rat Brain Acetylcholine after Acute Drug Administration

Treatment	Dose of narcotic (mg/kg)	No.	Brain ACh mean ± s.e. nmol g^{-1}	*P* value*
NaCl, 0.9%	—	8	18.7 ± 0.4	—
Nothing	—	8	18.1 ± 1.3	NS
NaBr i.v.t.	—	8	17.4 ± 0.9	NS
HC-3, 20 μg	—	11	9.7 ± 0.4	< 0.001
Acetylseco HC-3, 5 μg	—	8	10.6 ± 0.4	< 0.001
Morphine	10	8	19.1 ± 0.9	NS
Morphine	200	8	22.1 ± 0.5	< 0.01
Morphine + 20 μg HC-3	10	12	17.2 ± 1.0	NS
Nalorphine	20	8	18.8 ± 0.7	NS
Nalorphine + 20 μg HC-3	10	8	8.2 ± 0.1	< 0.001

* Group comparison Student "*t*" test to post-ether 0.9% saline treated rats.

Table 2 Effects of Morphine Withdrawal on Brain Acetylcholine after Chronic Administration of Morphine for 2 and 8 Weeks

Drug	Dose of drug (mg kg^{-1})	No.	Brain ACh mean ± s.e. (nmol g^{-1})	*P* value * A	B
2 week tolerant					
Morphine	200	9	16.6±0.4	NS	
Nalorphine	10	10	14.6±0.5	<0.01	
48 h withdrawn	0	9	14.4±0.4	<0.01	
Morphine+20 μg HC-3	200	6	11.4±0.3	<0.001	NS
Nalorphine+20 μg HC-3	10	8	7.9±0.2	<0.001	NS
48 h withdrawn+20 μg HC-3	0	7	8.0±0.3	<0.001	NS
Morphine+5 μg acetylseco HC-3	200	8	14.7±0.3	<0.01	<0.001
Nalorphine+5 μg acetylseco HC-3	10	10	7.8±0.1	<0.001	<0.001
48 h withdrawn+5 μg acetylseco HC-3	0	10	8.0±0.2	<0.001	<0.001
8 week tolerant					
NaCl, 0.9%	0	7	16.8±0.3	NS	
Nalorphine	50	8	15.8±0.5	NS	
48 h withdrawn	0	8	11.8±0.5	<0.001	
Nalorphine—10 min until death	50	7	14.4±0.4	<0.01	

* Group comparison Student "*t*" test. In column A treated rats are compared with acute 0.9% NaCl controls (data in Table 1) except for 8 week morphine tolerant animals which are compared with 8 weeks of 0.9% NaCl. In column B the values of brain ACh after narcotic plus ACh depletor are compared with the values after ACh depletor alone taken from Table 1.

were available as the Br salt, NaBr was used as a further control.

The mean brain ACh ± s.e. in nmol g^{-1} wet weight for various control procedures and treatments are given in Table 1. Control steady state brain ACh values for naive animals and animals treated with 0.9% NaCl were not significantly different. Both HC-3 and acetylseco HC-3 given i.v.t. reduced brain ACh. Morphine but not nalorphine prevented the reduction of brain ACh by HC-3. In doses of 10 mg kg^{-1}, i.p., neither affected steady state brain ACh.

The effects of giving morphine for either 2 or 8 weeks twice daily differed in several important ways from those obtained with single, acute injections. As shown in Tables 1 and 2, a single large dose of morphine caused a significant increase ($P<0.01$) in steady state brain ACh of normal animals, but not in 2 week tolerant rats. Nalorphine and 48 h abrupt withdrawal both significantiy ($P<0.01$) reduced steady state brain ACh. In 2 week tolerant rats, 200 mg kg^{-1} of morphine did not prevent depletion of brain ACh by HC-3 or acetylseco HC-3 as much as 10 mg kg^{-1} in non-tolerant rats did. Brain ACh was depleted even more during nalorphine and 48 h morphine withdrawal by 5 μg i.v.t. acetylseco HC-3 than in non-tolerant rats. In 8 week tolerant rats 48 h withdrawal and 10 min after nalorphine, there was a significant ($P<0.001$) decrease in steady state brain ACh. Although these data on steady state brain ACh disagree with those of Large and Milton[16,17], the findings of decreased utilization during acute morphine and enhanced utilization during withdrawal are in complete agreement. Different strains or the sex of the Wistar rats (female) may respond differently to enhanced brain ACh turnover, some showing an increase in steady state ACh and others a decrease. The interpretation of enhanced ACh turnover during morphine withdrawal using 5 μg i.v.t. acetylseco HC-3 depends on what comparison is made. In Table 1, acetylseco HC-3 reduced the ACh level from 18.7 (or 17.4) to 10.6, a mean reduction of 8.1 (or 6.8) nmol g^{-1}. In Table 2, this inhibitor reduced the ACh level from 14.6 (in the nalorphine group) to 7.8, a reduction of 6.8; and from 14.4 (48 h withdrawal) to 8.0, a reduction of 6.4 nmol/g.

The conclusion that ACh turnover is enhanced during morphine withdrawal is valid only if it is assumed that a comparison can be made between all groups and naive controls. In any event, it is quite clear that cholinergic brain function is altered by morphine in normal, tolerant and withdrawing animals. Any theory of morphine action must take into account these dramatic changes in brain ACh and hopefully point the way to more effective treatments of narcotic dependency.

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