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Behavioral Effects of Morphine on Free-Operant Avoidance Under Hyperbaric Pressure[1,2]

M. D. CURLEY, J. M. WALSH AND L. S. BURCH

Behavioral Sciences Department, Naval Medical Research Institute, Bethesda, MD 20014

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CURLEY, M. D., J. M. WALSH AND L. S. BURCH. *Behavioral effects of morphine on free-operant avoidance under hyperbaric pressure*. PHARMAC. BIOCHEM. BEHAV. 12(3) 413–417, 1980.—Morphine sulfate was tested under hyperbaric pressure to assess its effects on behavior. Four male hooded rats were trained to avoid brief electric shocks under a free-operant unsignalled avoidance procedure. Using an individual organism design, we injected each rat subcutaneously with morphine sulfate (2.0, 4.0, 6.0, 8.0 mg/kg body wt.) or saline (0.1 ml/100 g body wt.). Rats were tested at 1.0 and 7.1 atmospheres absolute (ATA) in a dry hyperbaric chamber while breathing a mixture of helium and oxygen. Each session lasted 60 min. Overall, the analgesic effects of morphine at 1.0 and 7.1 ATA were found to be similar. Shock avoidance by a rat was found to be a monotonic function of the drug dose; the fewest shocks were associated with the 2.0 mg/kg dose. Increased pressure did not significantly affect the number of shocks received by a rat across doses. Total responding remained stable throughout the study, but the temporal pattern of responding was differentially influenced by drug dose.

Morphine Hyperbaric Behavior Avoidance Rats

BECAUSE of its effectiveness in selectively relieving pain without impairing other sensory modalities [5], physicians have used morphine extensively in the treatment of a variety of injuries, including those incurred while diving. Within the past few years, however, researchers have discovered that the behavioral effects of various pharmaceutical agents under hyperbaric pressure may differ from their effects at surface pressure. Among the substances found to vary in their effect on behavior as a function of increased pressure were d-amphetamine sulfate [9,16]. Delta^9-tetrahydrocannabinol [18], ethanol and pentobarbital [13].

Toxicological [1,8] and physiological [2] investigations into the effects of morphine under hyperbaric pressure have been conducted, but the behavioral changes associated with morphine administration have not yet been systematically examined. By measuring the reaction time of mice dropped on a hot plate, Greenbaum and Evans [4] determined that there was no difference in the analgesic potency of morphine in mice breathing air at the surface or in mice breathing heliox at a simulated depth of 600 feet of sea water (19.2 atmospheres absolute [ATA]). Tofano and De Boer [14] also tested morphine-injected rats breathing heliox under varying shock intensities at 11 and 21 ATA. Rats injected with 100 mg/kg morphine responded to lower shock intensities at increased pressures than did injected animals breathing air at surface pressures, which suggests that the analgesic properties of morphine are diminished at higher pressures of helium and oxygen.

At surface pressures, the behavioral effects of morphine sulfate on signalled, discrete-trial avoidance responding were studied by Verhave, Owen, and Robbins [15]. Compared to predrug control data, 7 of 8 rats injected with doses of 6.5, 10, and 20 mg/kg showed a decrement in avoidance responding. Nevertheless, the authors found considerable individual variability in maximal effect, onset, and duration of the drug, which emphasizes the importance of using a design whereby each organism acts as its own control.

The purpose of this research was to investigate the behavioral effects of a narcotic analgesic (morphine) under hyperbaric pressure. Specifically, we attempted to measure the behavioral effects of various dosages of morphine injected into rats tested on a free-operant avoidance schedule while at pressures of 1.0 and 7.1 ATA.

EXPERIMENT 1

METHOD

To determine a non-lethal dose range of morphine sulfate for this study, we gathered preliminary toxicology data. Whereas relatively small doses of morphine (i.e. 6.5 mg/kg body weight) have been shown to alter the avoidance behavior of rats at surface pressures [15], hyperbaric investigations of morphine toxicology in rats have typically employed doses ranging from 20 mg/kg to 100 mg/kg [2, 8, 14]. At surface pressures, the lethal dose for 50% survival of group (LD_{50}) in rats injected with morphine was reported to be 237 mg/kg [3]. Small [8] calculated the LD_{50} to be 66.5 mg/kg in rats breathing a helium-oxygen mixture at 19.2 ATA. There-

[1]A version of this paper was presented at the annual meeting of the Undersea Medical Society, Key Biscayne, FL, 1979.

[2]Requests for reprints should be sent to Michael D. Curley, Behavioral Sciences Department, Naval Medical Research Institute, Bethesda, MD 20014.

fore, our first experiment attempted to find an acceptable upper dose limit for the behavioral assessment presented in Experiment 2.

Subjects

Thirty-two male albino rats (NMRI; O(SD) Sprague-Dawley derived) were obtained from the Institute's rat colony. The rats were approximately 90 days old, and weighed between 177 and 250 g.

Apparatus

All testing was conducted in a Bethlehem hyperbaric chamber, which could withstand internal pressures of 1000 pounds per square inch (psi). The chamber had a volume of approximately 170 liters, a diameter of 38 cm, and was 107 cm long. The chamber was penetrated with a number of threaded openings to accommodate pressure-fitted connectors for gas supplies and programming/recording apparatus. The internal temperature of the chamber was thermostatically maintained at 25° ± 2°C by a system of heating and cooling coils. A 5-compartment rectangular wire cage was used to separate the rats during testing; each compartment measured 12.2×22.2×15.6 cm.

Morphine sulfate injection, U.S.P., was obtained from Wyeth Laboratories; sodium chloride injection, U.S.P. (sodium chloride 0.9% in water) was obtained from Travenol Laboratories.

Procedure

An sc injection of either 8.0 or 80.0 mg/kg morphine, or 0.08 or 0.8 ml/hectogram saline was administered to each rat before compression. After injection, each rat was placed in an individual compartment and observed for 30 min. Four morphine-injected rats and one saline control rat were tested on each dive. Compression procedures involved venting the chamber for 5 min with a gas mixture of 80% helium and 20% oxygen, followed by pressurization with 100% helium at a rate of 0.6 ATA/min to 7.1 ATA. Rats remained at 7.1 ATA for 60 min. Oxygen levels were monitored at 7.1 ATA and found to be 2.8%. Decompression procedures followed standard tables developed in this laboratory [12]. Upon completion of the simulated dive all rats were observed for vital signs, gross motor activity, and decompression sickness. Rats showing signs of decompression sickness were treated by recompression to 2.8 ATA for 15 min while breathing 100% oxygen.

RESULTS AND DISCUSSION

Five of eight rats (63%) injected with a dose of 80.0 mg/kg morphine were dead upon arrival at 1.0 ATA, whereas only 2 of 16 rats (13%) receiving an 8.0 mg/kg injection of morphine died. No saline injected animals died. Two rats that were alive upon reaching 1.0 ATA but were afflicted with decompression sickness (i.e. paralysis of hind limbs) were successfully treated by recompression therapy.

An examination of five of the rats dead at 1.0 ATA revealed massive bubble formation in the peripheral blood vessels in three animals, which suggests fatal cases of decompression sickness. No gross pathological abnormalities were seen in the remaining two rats. Based on the finding that two rats in the 8.0 mg/kg dose groups exhibited decompression sickness, our decompression procedure was modified to include a 5-min stop on 100% oxygen at 4 ATA. No further incidence of decompression sickness was observed during the study.

To assess the gross effects of morphine on motor activity, we injected two rats with 8.0 mg/kg and observed them for 90 min at 1.0 ATA. Motor coordination was not impaired. Animals receiving an 80.0-mg/kg dose displayed a complete loss of locomotion for a period of 2 hr post-injection. Based on the maintenance of motor activity and low fatality rate of the 8.0-mg/kg groups, we decided to limit our maximum dose to 8.0 mg/kg.

EXPERIMENT 2

METHOD

Having determined an upper dose limit in Experiment 1, we were prepared to investigate the behavioral and analgesic effects of morphine under increased pressure. For this purpose, a free-operant avoidance procedure was selected. This procedure was selected (a) to eliminate the possible interaction of morphine-induced epigastric distress with reinforcers on a food schedule, and (b) to address both the analgesic and accompanying behavioral effects of morphine to a painful stimulus (shock).

Subjects

Four naive male Long-Evans hooded rats, obtained from Blue Spruce Farms, served as subjects. Long-Evans rats replaced the NMRI Sprague-Dawley derived rats used in Experiment 1 because of recent evidence suggesting the NMRI strain was overly susceptible to chronic respiratory disease. All rats were approximately 60 days old at the start of the experiment. Each animal was housed individually and provided with continuous access to water. Food intake was restricted slightly to maintain body weights between 260 and 400 g, which were comparable to those previously reported under high-pressure situations [10,11].

Apparatus

Rats performed in a modified BRS/LVE rodent chamber, which measured approximately 29.6×25.0×26.0 cm. A Plexiglas partition was placed diagonally across the length of the chamber to reduce the amount of floor space available to the animal, and an additional Plexiglas panel was placed over the front wall of the cage. A single response lever, which was attached to a microswitch requiring 13 g of force to operate, was projected through the front panel 4 cm above the floor. Sixteen stainless steel grids made up the chamber floor.

During all control sessions the chamber was placed inside a BRS/LVE Model SEC-002 light- and sound-reducing enclosure equipped with a filtered ventilation system. Shock was delivered through alternately wired floor grids by means of a Grason-Stadler model E1064GS shock generator-scrambler. Programming and data collection were accomplished by means of a BRS/LVE Interact system run by a NOVA 1200 minicomputer. A Gerbrands cumulative recorder provided continuous records of each subject's ongoing behavior during all sessions. All experimental sessions were carried out with the rodent chamber mounted inside the hyperbaric chamber described in Experiment 1.

Procedure

Subjects were tested under a free-operant avoidance procedure [7]. Under this schedule, a fixed time interval occurs

TABLE 1

THE ORDER OF CONDITIONS FOR ALL RATS AT EACH DOSE LEVEL

Day	1	2	3	4	5	6 and 7	8	9	10	11	12
Condition	Baseline	Baseline	Drug	Baseline	Control		Baseline	Baseline	Drug	Baseline	Control
Gas	Air	Air	$He-O_2$	Air	$He-O_2$		Air	Air	$He-O_2$	Air	$He-O_2$
ATA	1.0	1.0	1.0	1.0	7.1		1.0	1.0	7.1	1.0	1.0

between the presentation of brief electric shocks in the absence of a lever-press response (shock-shock [S-S] interval) and each response postpones the next shock for a fixed period of time (response-shock [R-S] interval). In the present study S-S and R-S intervals were 5 and 20 sec, respectively. Responses in the presence of shock had no consequence. Experimental sessions were 1 hr/day, 5 days/week. Shock duration remained constant at 0.5 sec throughout the study.

Initially, the rats were adapted to the experimental chamber by placing them in the chamber for one full-hr session and half of the following session in the absence of shock. Shock intensity was then set at 1.6 mA for the remainder of this session as well as for the two sessions that followed. Thereafter, shock intensity was increased to 3.0 mA for the remainder of the study. Subjects were tested daily until shock frequencies and response rates stabilized (approx. 35–40 sessions).

Adaptation to the hyperbaric chamber and to increased pressure was accomplished by conducting several sessions with the rodent chamber placed inside the hyperbaric chamber at ambient pressure and, subsequently, at pressure levels of 19.2, 41.4, and 59.2 psi. Compression procedures were identical to those outlined in Experiment 1; during decompression the modified procedure was used, which included a 5-min stop on 100% oxygen at 4 ATA.

Dose-response curves were then obtained over a range of doses of morphine sulfate both at normobaric (1.0 ATA [14.7 psi]) and hyperbaric (7.1 ATA [103.8 psi]) pressures using a gas mixture containing approximately 80% helium and 20% oxygen at 1.0 ATA, and 97% helium and 3% oxygen at 7.1 ATA. Doses of morphine were arbitrarily determined and one dose level was administered to each animal twice within a 2-week period. Table 1 shows the order of conditions to which each animal was exposed within each 2-week period throughout the experiment. Morphine sulfate was dissolved in saline and administered subcutaneously in the following sequence: 2.0, 6.0, saline only, 4.0, and 8.0 mg/kg. All doses of the drug as well as those of saline were injected 30 min before the start of a session. The doses of morphine are expressed as the salt. Drug solutions were mixed individually so that a constant volume of solution was administered across doses (0.1 ml per hectogram body wt.).

RESULTS

Shock rate (total session shocks/60) was used to assess the pain-relieving properties of the drug. Response rate (total session responses/60) served as an indicator of behavioral change (e.g. mental clouding, drowsiness, mood alteration) that may accompany morphine administration [5]. A repeated measures analysis of variance technique [6] was used to test for significance. Graphed data represent group means and are representative of individual performance unless otherwise noted.

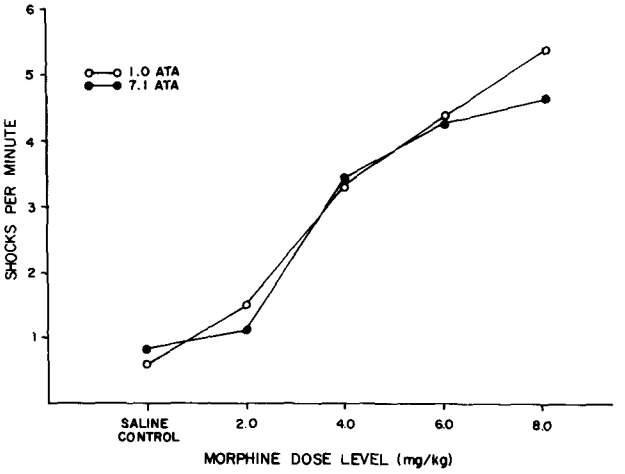

FIG. 1. Mean number of shocks received per minute as a function of drug dose and pressure level. Standard errors ranged from 0.19 (saline at 1.0 ATA) to 1.99 (8.0 mg/kg at 7.1 ATA).

Shock Rate

The analgesic effectiveness of morphine sulfate as reflected by shock rate was directly related to dose magnitude; as dosage increased, there was a concomitant increase in the number of shocks received ($F=4.94, p<0.025$). Moreover, as clearly shown in Fig. 1, this relationship was maintained at both surface (1.0 ATA) and hyperbaric (7.1 ATA) pressures. There was no significant effect of increased pressure on shock rate across drug doses; indeed Fig. 1 shows that the mean shock rates at each dose for 1.0 and 7.1 ATA were virtually identical. The divergence in shock rate seen at the 8.0-mg/kg dose primarily reflects a sharp increase in the number of shocks received by one rat (no. 157) at 1.0 ATA. No statistically significant differences in shock rate were observed during baseline air or baseline $He-O_2$ control sessions as a function of the time course of the study.

The analgesic time course of the drug was assessed by recording the number of shocks received by each rat at 20, 40, and 60 min into the test session. These points correspond to 60, 80, and 100 min postinjection, respectively. No significant differences were found among the 20-min segments, which suggest that the peak effectiveness of the drug dose may have been reached at, or prior to, 20 min into the test session.

Response Rate

Group response rate was not significantly affected by pressure level or drug dose. As can be seen in Fig. 2, stable responding was maintained throughout the study except for a decline under the 8.0-mg/kg dose at 1.0 ATA. This reduction

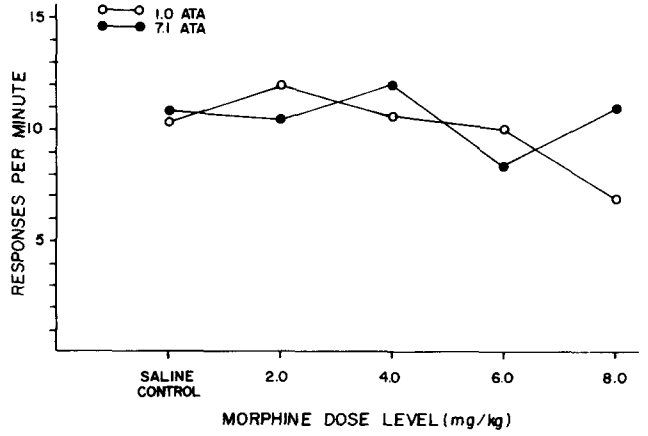

FIG. 2. Mean number of responses made per minute as a function of drug dose and pressure level. Standard errors ranged from 0.75 (saline at 7.1 ATA) to 2.72 (6.0 mg/kg at 7.1 ATA).

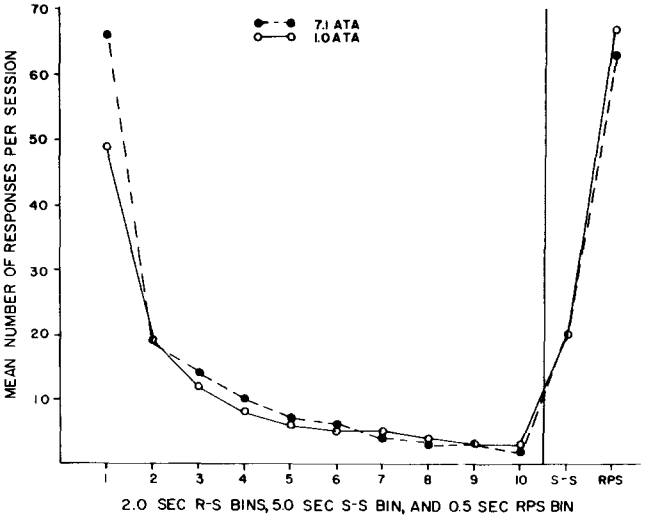

FIG. 4. Distribution of responding within response-shock (R-S), shock-shock (S-S), and responses in the presence of shock (RPS) intervals as a function of pressure level; data is combined across morphine and saline doses.

in response rate resulted from Rat no. 157 receiving many shocks while emitting few responses. Within-subject response performance, however, showed individual variation across doses. For example, Fig. 3 presents cumulative records for Rat no. 157 across drug doses and pressures. Systematic increases in shock rate and decreases in response rate can be seen to accompany increases in drug dose, but not in pressure level.

Analysis of the temporal patterning of responding across doses revealed that frequency of responding was greatest during shock presence (RPS) and in the 2 sec immediately following shock offset (bin 1), as shown in Fig. 4. A gradual reduction in response frequency then occurred over the remaining portion of the R-S interval (bins 2–10), followed by an increase in responding during the S-S interval. Although no statistically significant differences in this response pattern were associated with pressure level, considerably more responses were recorded in the first bin at 7.1 ATA than at 1.0 ATA.

The distribution of responses in the R-S, S-S, and RPS

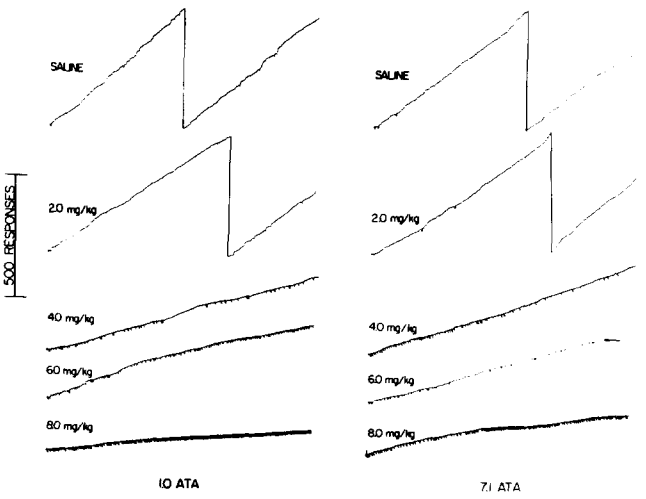

FIG. 3. Cumulative records of Rat. no. 157 at each drug dose and pressure level. Each downward deflection of the pen indicates a shock delivered.

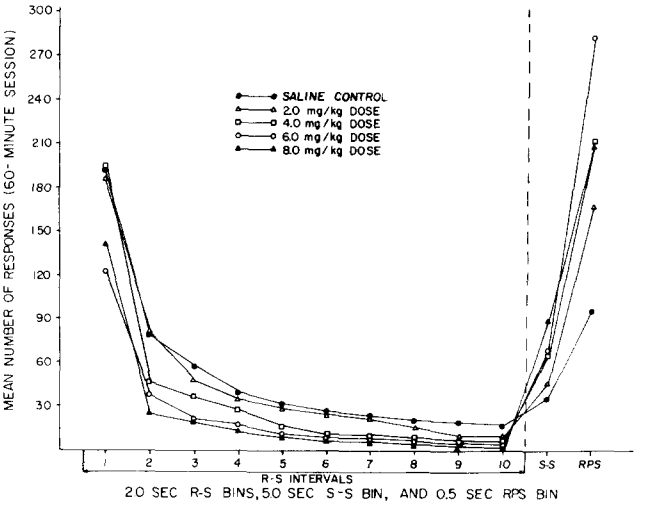

FIG. 5. Distribution of responding within response-shock (R-S), shock-shock (S-S), and responses in the presence of shock (RPS) intervals as a function of drug dose.

bins was substantially affected by drug dose ($F=2.18$, $p<0.001$). Higher doses of the drug (6.0 and 8.0 mg/kg) tended to yield greater numbers of responses in the S-S and RPS bins and fewer responses in the first 2-sec bin than did the lower morphine doses and saline control. This relationship is illustrated in Fig. 5. A clear dose-response relationship is evident over R-S bins 2 through 10, in that the frequency of responding within bins 2–10 was greatest under the control dose, and least under the 8.0 mg/kg dose.

No differences or shifts in response distribution were found during baseline control sessions with either air or He-O_2 as the breathing gas. All subjects survived the experiment without apparent ill effects, and were still living 6 months after the last dive. No cases of decompression sickness were observed.

DISCUSSION

The finding that similar analgesic dose-response functions were evident at both 1.0 and 7.1 ATA is significant. Although

this result may appear at odds with the findings of Tofano and De Boer [14], a further examination of their data reveals no substantial differences in analgesia at 1.0, 11.0, or 21.0 ATA *1 hour* after administration. In other words, the *peak effectiveness* of the drug does *not* differ with increased pressure; however, there are significant differences in the metabolism and excretion of morphine sulfate as a function of pressure after the peak effect is reached. Thus, by using a more sophisticated operant technique we have clarified and supported the findings of Tofano and DeBoer [14], and extended the data base to 7.1 ATA.

Behaviorally, the finding that increased hyperbaric pressure did not significantly alter either the group rate or distribution of lever-pressing is noteworthy. This finding suggests that the cognitive abilities used in effective shock avoidance were not substantially affected at 7.1 ATA in the absence of the drug. Whereas the group rate of lever-pressing remained stable across doses and pressure level, the spacing of responses within the R-S, S-S, and RPS intervals changed as a function of drug dose. It was reasoned that as higher doses of morphine were administered to the rat, the perceived aversiveness of the nociceptive stimulus electric shock and resulting physiological arousal would decrease. This reduction would lead the rat to emit fewer responses as the time since the last shock increased. The end result of this cycle should be that the rat would receive more shocks within a one-hour session because of a decrease in response frequency in the latter portions of the R-S interval. Our results support this reasoning. As the dosage increased, more responses were made in the presence of shock and during the S-S interval than at the lower doses, with fewer responses emitted during the R-S interval. This shift in responding resulted in more shocks being delivered at the higher doses of the drug, for on this Sidman avoidance schedule the optimal strategy was to emit a response every 18–19 sec into the R-S interval. Thus, increasing the dose given to the rats appears to have increased analgesia and affected the rats' capacity to effectively discriminate time, yet did not significantly impair their ability to physically emit a lever-press response.

This study was conducted using a helium-oxygen mixture as the breathing gas under pressure. Compressed air was avoided as the breathing medium because of the possible presence of nitrogen narcosis at a pressure of 7.1 ATA. Previous work in this laboratory has demonstrated that adaptation to nitrogen narcosis is possible [17]; however, different subjects adapt at varying depths. The authors therefore sought to avoid this possible confound by using a helium-oxygen mixture. Further, Navy saturation divers normally use a helium-oxygen gas mixture as the breathing medium. In a U.S. Navy saturation dive, therefore, it is probable that morphine administration to relieve pain associated with trauma would occur in a helium-oxygen environment.

The generalizability of the present findings to other diving situations using different breathing gases at varying depths remains to be tested. Pharmacological investigations at pressures of 2.8 and 6.0 ATA from the air treatment table would appear to be of particular interest.

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The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, DHEW, Pub. No. (NIH) 78–23.

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