Pharmacology Biochemistry & Behavior, Vol. 22, pp. 921–931, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Indomethacin Facilitates Acute Tolerance to and Dependence Upon Morphine as Measured by Changes in Fixed-Ratio Behavior and Rectal Temperature in Rats[1]

JANN A. NIELSEN[2,3] AND SHELDON B. SPARBER

Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455

Received 26 June 1984

NIELSEN, J. A. AND S. B. SPARBER. *Indomethacin facilitates acute tolerance to and dependence upon morphine as measured by changes in fixed-ratio behavior and rectal temperature in rats.* PHARMACOL BIOCHEM BEHAV 22(6) 921–931, 1985.—The effects of indomethacin, a prostaglandin (PG) synthetase inhibitor, on acute tolerance to and dependence on morphine were investigated. Twelve mature, male Long-Evans rats were trained to lever press for food reinforcement on a fixed-ratio 30 schedule (FR 30 behavior) and have their rectal temperature taken. The experimental protocol began with taking the rat's temperature followed by a 30 minute behavioral session. Immediately after this session the animal was injected with indomethacin or its vehicle. Two-and-a-half hours later this procedure was repeated, except that morphine or saline was administered. After an additional 2.5 hours had elapsed, a 60 minute behavioral session occurred. Half-way through the session the rat was injected with morphine (tolerance), naloxone (dependence), or saline. Immediately after the session the rat's temperature was recorded. Indomethacin potentiated the acute tolerance to the behavioral suppressant and hyperthermic effects of morphine. Indomethacin pretreatment also greatly enhanced the capacity of naloxone to decrease temperature and suppress FR 30 behavior in morphine-treated rats. These effects were not due to indomethacin altering the acute effects of morphine or the amount of morphine in the brain. These data suggest that indomethacin is inhibiting synthesis of PGs which are important in morphine tolerance and dependence.

Prostaglandins Morphine Tolerance Dependence Operant behavior Body temperature Rats

THE accompanying paper suggests that central administration of prostaglandin E_2 (PGE_2) facilitates, while $PGF_{2\alpha}$ attenuates acute dependence upon morphine in rats [28]. Altering the ratio of PGs in the central nervous system (CNS) might, therefore, alter morphine dependence. Indomethacin inhibits PG synthetase, but also increases the ratio of $PGE_2/PGF_{2\alpha}$ in rat brain [1,16]. Therefore, indomethacin might potentiate naloxone's effects in morphine-treated rats.

The adaptive processes related to the development of dependence on opiates are closely related to the development of tolerance to opiates. PGs might also be involved in opiate tolerance since tolerance develops to methionine-enkephalin-induced production of PGE-like material in rat brain [34]. By inhibiting PG synthesis, indomethacin might potentiate the development of tolerance to opiates.

Morphine tolerance [2, 3, 18, 32] and dependence [2, 3, 18, 19, 39] have been shown using operant behavior measures. Tolerance to and dependence on relatively low doses of morphine can be shown by operant behavioral measures in rats as soon as 2 to 3 hours after a single dose of morphine [25,36]. The single-dose tolerance/dependence model enables the investigator to circumvent many of the secondary and tertiary consequences of multiple injections or pellet implantation methods, including overdosing, toxicity, multiple withdrawal episodes, variable absorption rates, etc. Therefore, the acute model was used to determine if indomethacin affects the adaptive processes related to opiate administration.

Body temperature has also been used to measure morphine tolerance and dependence. Acute administration of low doses of morphine increase the body temperature of rats [21]. Tolerance to this effect of morphine in rats has been shown in some [8, 15, 20, 27, 33], but not all experiments [24,29]. Dependence on morphine can be shown by a naloxone-induced decrease in body temperature in chronic morphine-treated rats [27, 29, 33]. Mucha and coworkers [27] have studied several measures of morphine tolerance and dependence and conclude that temperature is a suitable measure of both phenomena in rats. However, acute tolerance or dependence, as measured by changes in tem-

[1]Supported in part by USPHS grants MH 08565 and DA 00532.

[2]Present address: Department of Pharmacology, Northeastern Ohio Universities College of Medicine, 4209 State Route 44, Rootstown, OH 44272.

[3]Requests for reprints should be addressed to J. A. Nielsen at present address.

perature, after a single dose of morphine, have never been reported. Therefore, we determined if this could be demonstrated and if indomethacin would alter its development or expression, especially in light of the fact that PGs are involved in several forms of hyperthermia [13, 26, 30] and PG synthesis inhibitors are effectively used to suppress hyperthermias mediated by the release, *in vivo*, of PGs [14].

We found that indomethacin altered the effects of morphine. It was possible that this effect was due to indomethacin altering the penetration of morphine into the central nervous system. Therefore, experiments were performed to determine if indomethacin significantly altered the concentration of morphine in the brains of rats at times when we observed changes in responsiveness to morphine as a consequence of indomethacin pretreatment.

METHOD

DRUGS

Indomethacin (Sigma Chemical Company, Saint Louis, MO) was dissolved in isotonic saline with the aid of sodium carbonate and shaking. The pH of the solution was adjusted to 7.5 by addition of hydrochloric acid. Morphine sulfate (S.B. Penick Company, New York, NY) and naloxone hydrochloride (generously supplied by Endo Laboratories, Garden City, NY) were dissolved in saline. To determine the brain levels of morphine, 10 μg [(1 (n)-^{3}(H) morphine/kg (Spec. Act. 28 Ci/mmol, Amersham Corporation, Arlington Heights, IL)] plus 3.75 mg morphine/kg were injected in Experiment 6. All solutions were prepared daily and injected (IP) in a volume of 1 ml/kg of body weight. Drug doses are expressed as the free base.

EXPERIMENTS 1–4

Eighteen drug-naive, male Long-Evans rats (Simonsen, Gilroy, CA) were used in this study. They were food deprived to approximately 80% of their free-feeding weights and trained to lever press on a fixed-ratio (FR) 30 schedule for food reinforcement. Between experiments the animals were allowed free access to food. A computer-based Interact (BRS/LVE, Beltsville, MD) system was programmed to control environmental contingencies and record behavior and reduce it to the number of reinforcers earned and the responses emitted by the rat during each minute. A record of each session was also obtained on cumulative recorders (R. Gerbrands Company, Arlington, MA).

When all of the rats were lever pressing on a FR 30 schedule they were habituated to being injected (IP) with a 0.9% saline solution and having their rectal temperature taken. Temperatures were determined by inserting a temperature probe (Yellow Springs Instrument Company, Yellow Springs, OH) 5 cm into the rat's rectum, taping it to the tail, returning the rat to its home cage, and recording its temperature 3 minutes later from a telethermometer (Yellow Springs Instrument Company). The temperature was then recorded and the probe removed.

Experiments in which only indomethacin vehicle and saline was injected [nondrug experiments—vehicle, saline, saline (see Figs. 1, 2, and 5)] were performed for 9 days at which time the rats' behaviors and temperatures appeared stable. Stability in the behavioral and rectal temperature measurements was evidenced by a coefficient of variability of less than 10% and 2%, respectively. Nondrug experiments were performed between experiments 1, 2, 3, and 4 to show that the rats' behavior and temperature were not permanently altered by the drugs used in these experiments.

TABLE 1
EXPERIMENTAL PROTOCOL

Time (min)	Event
0	Insert rectal temperature probe. Return rat to home cage.
3	Record temperature. Remove probe. Place rat in operant chamber. Start behavioral session one.
33	Stop behavioral session. Give injection (saline, indomethacin vehicle, or indomethacin). Return rat to home cage.
180	Insert rectal temperature probe. Return rat to home cage.
183	Record temperature. Remove probe. Place rat in operant chamber. Start behavioral session two.
213	Stop behavioral session. Give injection (saline or morphine). Return rat to home cage.
363	Place rat in operant chamber. Start behavioral session three.
393	Give injection (saline, morphine or naloxone). Start behavioral session four.
423	Stop behavioral session. Insert rectal temperature probe. Return rat to home cage.
426	Record temperature. Remove probe. Return rat to home cage.

Experiment 1—Choosing an Appropriate Dose of Indomethacin

This experiment was carried out to determine the highest dose of indomethacin which had no significant effect on FR 30 behavior or rectal temperature. In this and the following 3 experiments the same general protocol was used (Table 1). After the rat's temperature was determined, each animal was placed (individually) within an operant chamber and allowed access to food reinforcers for 30 minutes on the FR 30 schedule (behavioral session 1). Immediately after this initial behavioral session the rat was injected with either saline, indomethacin or its vehicle. Two-and-a-half hours later the procedure was repeated (behavioral session 2), except that saline or morphine was administered. After an additional 2.5 hours had elapsed, the rat was again placed within the operant chamber, this time for two consecutive 30-minute behavioral sessions (3 and 4). Between behavioral sessions 3 and 4 the rat was injected with either saline, morphine (tolerance) or naloxone (dependence). Temperature was recorded im-

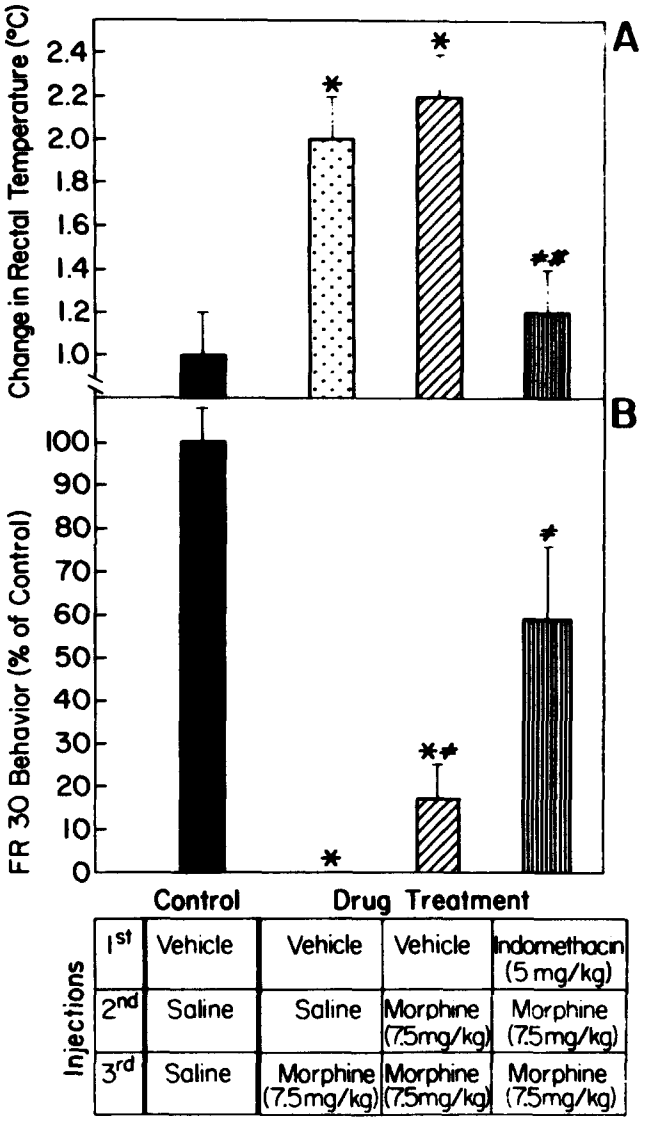

FIG. 1. Acute tolerance to the hyperthermic and behavioral suppressant effects of morphine after pretreatment with indomethacin. *$p<0.05$ compared with the same rats treated with vehicle, saline and saline (2-tailed, paired Student t-test). $p<0.05$ compared with the appropriate group treated with vehicle, saline and morphine (≠) or vehicle, morphine and morphine (#) (analysis of variance and Duncan's new multiple range test). Injections were 3 hours apart. The 12 rats used in the control part of the experiment were divided into the drug treatment groups. *Panel A*—Rectal temperature recorded 30 minutes before the first injection was subtracted from the temperature recorded 30 minutes after the last injection. Their temperature before the first injection was 37.4±0.2°C. *Panel B*—Data represents FR 30 behavior during a 30-minute session after the last injection. Their response rate was 1.80±0.14 responses/second during the control portion of the experiment. All values are mean±S.E.

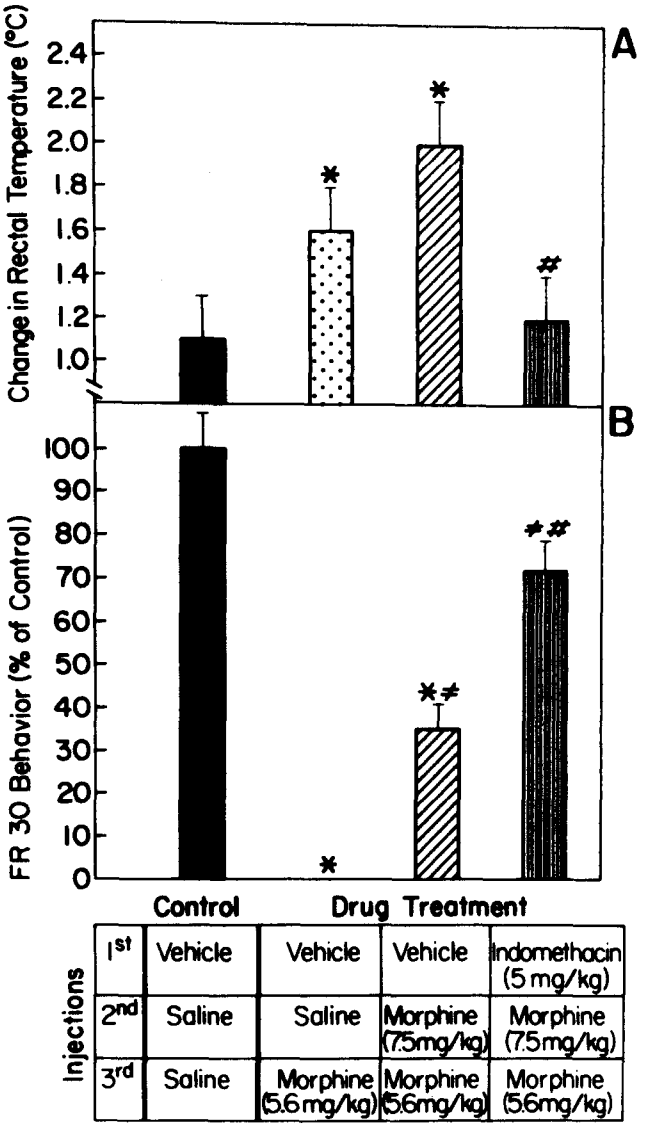

Fig. 2. Acute tolerance to the hyperthermic and behavioral suppressant effects of morphine after pretreatment with indomethacin. *$p<0.05$ compared with the same rats treated with vehicle, saline and saline (2-tailed, paired Student t-test). $p<0.05$ compared with the appropriate group treated with vehicle, saline and morphine (≠) or vehicle, morphine and morphine (#) (analysis of variance and Duncan's new multiple range test). See Fig. 1 for an explanation of the data presentation. *Panel A*—The rats' mean temperature before the first injection was 37.1±0.3°C. *Panel B*—Their response rate was 1.71±0.14 responses/second during the control portion of the experiment. All values are mean±S.E.

mediately after behavioral session 4. Experiments were performed at approximately the same time each day.

Indomethacin vehicle was administered to all the rats and was found to have no effect on behavior or temperature. The rats were then divided into 2 groups, using a block design in which the 2 fastest responders were randomly assigned to one or the other treatment dose, followed by the next 2 fastest responders, etc. They were then injected with 10 or 20 mg indomethacin/kg. Both doses suppressed behavior and decreased temperature (vide infra). In addition, 4 of the 9 rats receiving the higher dose of indomethacin died. Two others were not used in the remaining experiments because their operant behavior was suppressed for days.

Five days later the remaining 12 rats were found to have

recovered from the effects of indomethacin. They were again randomly divided into 2 groups and administered 2.5 or 5 mg indomethacin/kg. Five mg/kg was the highest dose which had no effect on behavior or temperature (vide infra) and therefore, was the dose used in subsequent experiments.

EXPERIMENTS 2 AND 3–ACUTE TOLERANCE TO MORPHINE

Experiment 2

The 12 rats used in experiment 1 were randomly divided into 3 groups and administered vehicle, saline, and morphine; or vehicle, morphine and morphine; or indomethacin, morphine and morphine 1 week after the termination of experiment 1. All injections of the opiate were at a dose of 7.5 mg/kg. Since the second and third group were given a second dose of morphine to determine if tolerance had developed, the first group was administered 7.5 mg morphine/kg 3 hours after the third injection so that all rats received the same amount (15 mg/kg) of morphine per day. This was an attempt to control for any potential morphine carryover effect to subsequent experiments on succeeding days.

Acute tolerance to the FR 30 behavior suppressant or hyperthermic effects of the relatively high dose (7.5 mg/kg) of morphine used in the first experiment was not observed. Complete tolerance to the operant behavioral suppressant effect of the narcotic has previously been demonstrated when the priming dose (second injection) was 7.5 mg/kg and the challenge dose was 3.75 mg/kg [36]. A combination of doses which produce evidence of partial tolerance was needed in order to determine if indomethacin would attenuate or potentiate this adaptive phenomenon. Therefore, the challenge dose was lowered to 5.6 mg/kg in experiment 3.

Experiment 3

A week later the rats were randomly reassigned to treatment groups and experiment 2 was partially replicated. The only difference between this experiment and the previous one was that the challenge dose of morphine was decreased to 5.6 mg/kg.

In order to control for dissimilar drug histories, the group given only one injection of morphine (5.6 mg/kg) during the experiment was administered an additional dose of 7.5 mg/kg 3 hr after the experiment.

Experiment 4—Acute Dependence of Morphine

A week later the 12 rats used in the previous experiments were again randomly divided into 2 groups and administered vehicle, saline and naloxone (2.5 mg/kg), or indomethacin, saline and naloxone (2.5 mg/kg). It was determined that 5 mg indomethacin/kg or its vehicle, followed 6 hours later by 2.5 mg naloxone/kg had no effect on the rats' FR 30 behavior or rectal temperature (vide infra).

One week after the above manipulation the 12 rats were randomly divided into 3 groups and administered vehicle, morphine and saline; or vehicle, morphine and naloxone; or indomethacin, morphine and naloxone. In this manner one could determine if the rats were acutely dependent on morphine and if indomethacin affected acute dependence. The doses of morphine (15 mg/kg) and naloxone (2.5 mg/kg) and the interval between them, were chosen because acute dependence on morphine has previously been shown [36] using these values.

Experiment 5—Indomethacin's Effect on Morphine-Induced Hyperthermia

Indomethacin diminished the increase in temperature recorded after the challenge dose of morphine in experiments 2 and 3. We were unable to determine, because of the experimental design, if this resulted from indomethacin reducing the initial effect of morphine. Experiment 5 was performed to determine if this was the case.

Twelve different drug-naive, mature, male Long-Evans rats (Simonsen) were habituated to an injection of a 0.9% saline solution and the measurement of rectal temperature as described above. The rats were slowly food-deprived to approximately 80% of their free-feeding weights (400–450 g) to control for body weight factors.

Nondrug experiments were performed for 11 days, until the rats habituated to being handled, etc., and their temperatures became stable. The experimental protocol began by determining the rats' temperatures followed immediately by an injection (IP) of indomethacin or its vehicle. A second injection of saline or morphine (7.5 mg/kg) was made 3 hours later and the rats' rectal temperatures were determined 30 minutes later. All the rats received vehicle and saline, followed two days later by indomethacin and saline. One week later the rats were randomly divided into 2 groups; half were administered indomethacin and morphine, the other half vehicle and morphine.

Experiment 6—Indomethacin's Effect Upon the Amount of Morphine in Rat Brain

Experiment 6 was performed to determine if indomethacin's facilitation of tolerance to and dependence upon morphine could be due to indomethacin altering the amount of morphine in the brain. Twenty-four different drug-naive, male Long-Evans rats (Simonsen) were gradually food-deprived to 80% of their free-feeding weights, habituated to an injection of 0.9% saline, and then randomly divided into 6 groups of 4 rats each. Indomethacin or its vehicle was administered 180 minutes before morphine (3.75 mg/kg plus 10 μg of 1 (n)-^{3}(H) morphine/kg), and the rats were killed 20, 30, or 50 minutes after morphine injection, respectively. After decapitation, the brains were removed and assayed for morphine by modifications of the method of Tulunay and coworkers [42]. The modifications involved homogenizing the brain in 2 volumes of distilled water, adding an equal volume of 0.5 M glycine buffer (pH 9), and extracting twice with 20 ml portions of 10% ethanol in chloroform.

DATA ANALYSIS

Where appropriate, data were analyzed by the paired Student t-test, with each rat serving as its own control, or a group Student t-test, to determine if a treatment altered the parameter under study [36]. A one-way analysis of variance was used to determine if any of the 3 treatments used in experiments 2, 3, and 4 altered behavior or temperature. Significant differences between treatment means were determined by Duncan's new multiple range test. All data are expressed as the mean plus or minus one standard error of the mean.

RESULTS

EXPERIMENTS 1–4

Nondrug Experiments

Before the first drug experiment, the rats used in the first

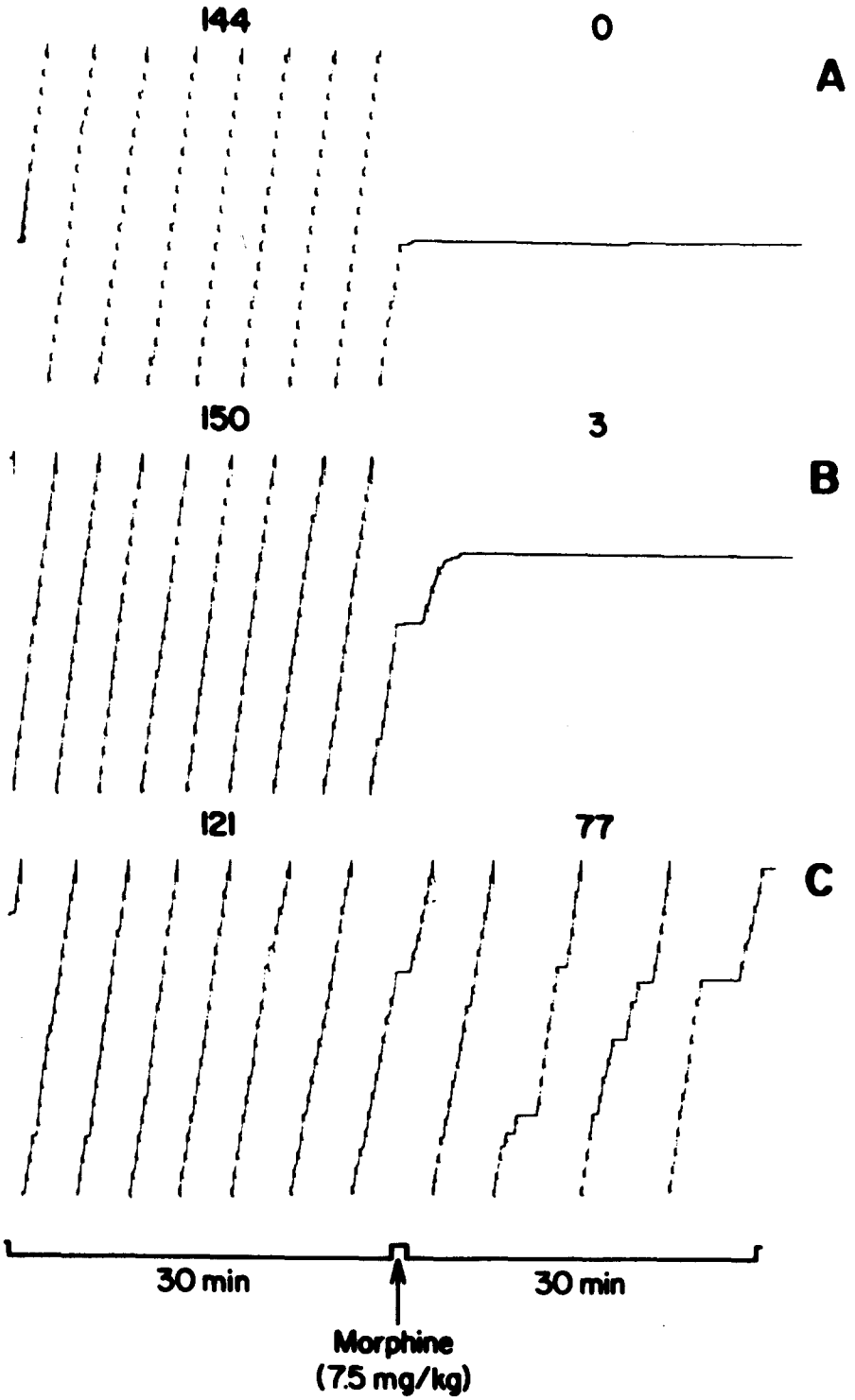

FIG. 3. Sample cumulative records showing that indomethacin pretreatment augmented tolerance to morphine's behavior suppressant action. Rats were injected 3 times at 3-hour intervals: rat 58 *(Panel A)* with vehicle, saline, and morphine; rat 46 *(Panel B)* with vehicle, morphine, and morphine; and rat 52 *(Panel C)* with indomethacin (5 mg/kg), morphine, and morphine. FR 30 behavior recorded on the cumulative records is shown for 30-minute periods before and after the third injection. All morphine injections were 7.5 mg/kg. Values above the cumulative records represent the number of reinforcers earned by the rats during the sessions depicted. Responding rate is reflected by the slope of the recording. Delivery of a reinforcer is indicated as a pip on the ascending record. Behavior during the 30-minute period before the third injection is not different from control behavior, e.g., rats 46 and 52 had recovered from the suppression of behavior caused by the first administration of morphine.

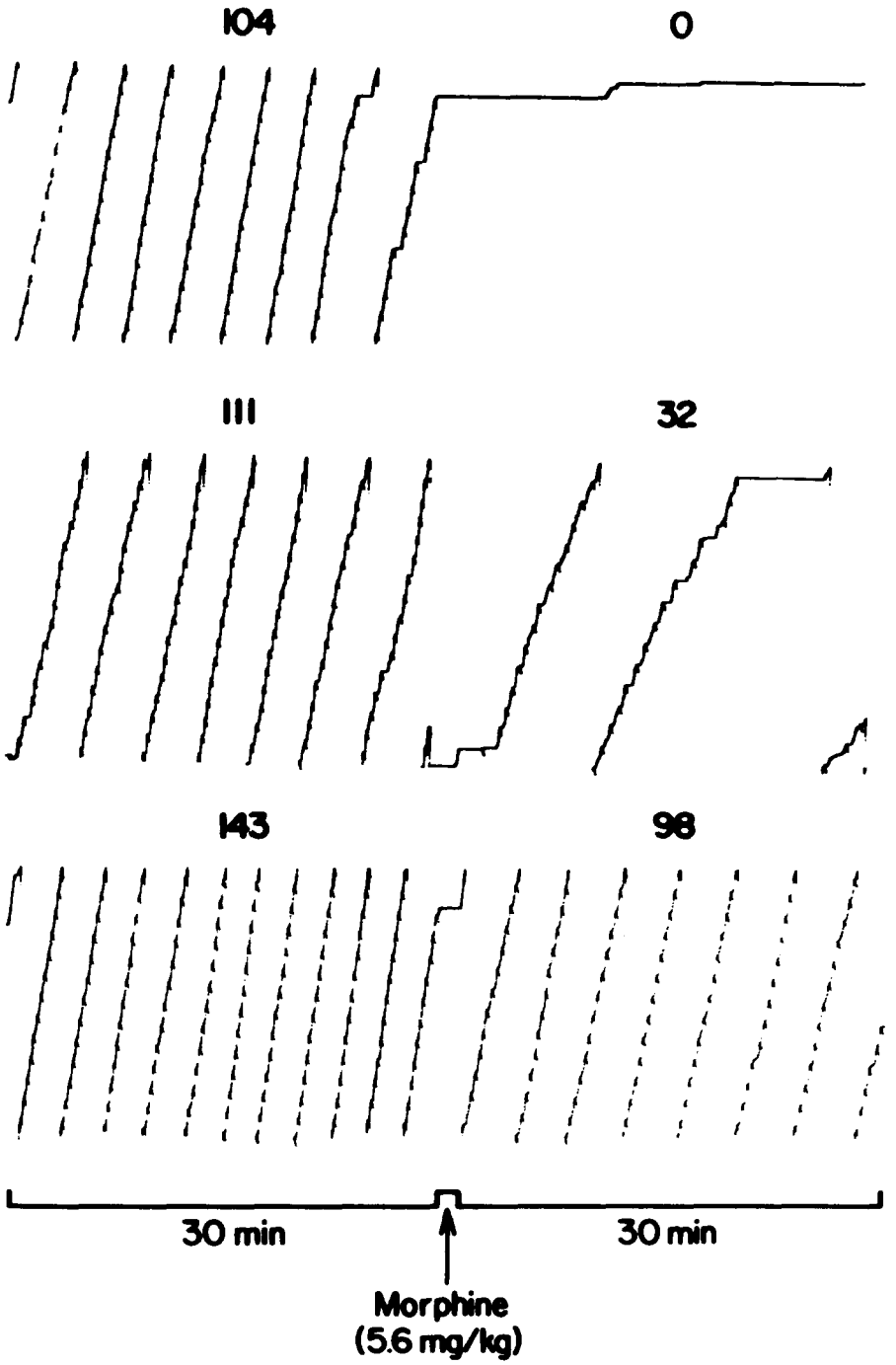

FIG. 4. Sample cumulative records from individual rats demonstrating tolerance to morphine-induced suppression of behavior and enhancement of this tolerance by pretreatment with indomethacin. The experimental design is the same as described in Fig. 3 legend, but with the challenge dose (third injection) lowered to 5.6 mg morphine/kg. Rat 52 *(Panel A)* was injected with vehicle, saline and morphine; rat 47 *(Panel B)* with vehicle, morphine, and morphine; and rat 51 *(Panel C)* with indomethacin (5 mg/kg), morphine, and morphine. Behavior during the 30-minute period before the third injection is not different from control behavior, e.g., rats 47 and 51 had recovered from the suppression of FR 30 behavior by the first administration of morphine. See Fig. 3 legend for an explanation of the values above the cumulative records, and of recording slope and pips.

four experiments had an average rectal temperature of 37.5±0.2°C. Their temperature 3.0 hours later was 37.8±0.2°C. During behavior sessions 3 and 4 a significant elevation in temperature was observed (38.5±0.2°C). The FR 30 lever-pressing behavior for all rats during the last four experiments, before drug administration was initiated, was 1.88±0.12, 1.86±0.08, 1.86±0.14, and 1.65±0.16 responses per second, for behavioral sessions 1 through 4, respectively.

There was no systematic baseline shift in their temperatures or response rates throughout the course of experimentation. This indicated that the different drugs did not permanently alter or have a carryover effect on the rats' temperatures or behaviors. The experimental groups did not differ from each other in terms of their temperature or behavior during experiments when saline was injected. Responding was significantly lower in the fourth behavioral session, probably due to food satiation. Therefore, experimental behavioral data is presented in terms of a percentage of the response rate of individual rats during the corresponding behavioral session of the most recent nondrug experiment performed on a previous day.

Experiment 1—Choosing an Appropriate Dose of Indomethacin

Indomethacin (2.5 and 5 mg/kg) and its vehicle had no effect on the rats' FR 30 behaviors or rectal temperatures in any experiment reported herein. Ten mg indomethacin/kg produced diarrhea, significantly suppressed behavior 5.5 to 6 hours (56±6% of control) and 6 to 6.5 hours (36±8% of control), and decreased temperature 7 hours (0.8±0.2°C below control) after administration. Twenty mg/kg had similar effects, and in addition, 4 of 9 rats died from 2 to 12 days after injection of this dose. In a separate study, 6 of 10 rats administered 28.8 mg indomethacin/kg died within 12 days (unpublished observations).

Experiments 2 and 3—Acute Effects of Morphine

Morphine, at doses of 5.6 and 7.5 mg/kg, significantly increased temperature to 0.5 and 1.0°C, respectively, above control values 30 minutes after administration (Figs. 1 and 2, panel A). These doses of morphine eliminated operant behavior during the 30-minute session immediately following its administration (behavioral session 4) (Figs. 1 and 2, panel B). Behavior 2.5 to 3 hours after morphine (7.5 or 15 mg/kg) (behavioral session 3) was not different from control and was not altered by pretreatment with indomethacin. Therefore, when the second dose of morphine was given, the subjects were emitting behavior at control rates.

Effects of Indomethacin on Acute Tolerance to Morphine

If the rats were pretreated with indomethacin and morphine (7.5 mg/kg), temperature after the challenge dose of morphine was significantly lower than the other groups given morphine 7.5 mg/kg and 5.6 mg/kg, $F(2,9)=4.31$, $p<0.05$, or 7.5 mg/kg and 7.5 mg/kg, $F(2,9)=6.17$, $p<0.02$ (Figs. 1 and 2, panel A).

Acute tolerance to the behavioral suppressant effects of morphine was made evident or obviously enhanced when rats were pretreated with indomethacin and morphine (7.5 mg/kg) followed by a challenge dose of 7.5 and 5.6 mg morphine/kg, respectively. As is shown in Fig. 1 (panel B), only the rats administered indomethacin plus morphine responded during behavioral session 4 significantly above zero rates when challenged with a second injection of 7.5 mg morphine/kg, $F(2,9)=4.94$, $p<0.05$. When the challenge dose of morphine was reduced to 5.6 mg/kg (Fig. 2, panel B), tolerance was evident without prior treatment with indomethacin, $F(2,9)=18.30$, $p<0.001$. In addition, a significant enhancement of tolerance was obtained in the group pretreated with the PG synthetase inhibitor and challenged with 5.6 mg morphine/kg.

Figures 3 and 4 show sample cumulative records from behavioral sessions 3 and 4 from individual rats demonstrating tolerance to morphine-induced suppression of behavior and the enhancement of this tolerance by indomethacin.

Experiment 4—Acute Dependence on Morphine

Indomethacin pretreatment greatly enhanced the capacity of naloxone to decrease temperature, $F(2,9)=12.37$, $p<0.003$ (Fig. 5, panel A) and suppress behavior during session 4, $F(2,9)=13.65$, $p<0.002$ (Fig. 5, panel B) in morphine-treated rats. Morphine's (15 mg/kg) behavioral suppressant effect was not evident 2.5 to 3 hours after injection during behavioral session 3. Naloxone had no effect on behavior or temperature except when administered 3 hours after morphine at which time it significantly suppressed behavior during session 4 and decreased temperature. Naloxone and indomethacin had no effect on behavior or temperature, but indomethacin significantly potentiated naloxone's effect.

Figure 6 shows sample cumulative records from behavioral session 4 from individual rats demonstrating the naloxone-induced decrease in FR 30 behavior in morphine-treated rats and the potentiation of this effect by indomethacin.

Experiment 5—Indomethacin's Effect on Morphine-Induced Hyperthermia

Indomethacin pretreatment did not alter morphine-induced hyperthermia (Table 2).

Experiment 6—Indomethacin's Effect Upon the Amount of Morphine in Rat Brain

Indomethacin did not alter the amount of morphine in brain 20, 30, or 50 minutes after injection of the opiate (Table 3). The radioactivity in brain extract from rats injected with (^{3}H)-morphine cochromatographed with authentic (^{3}H)-morphine. Recovery of added morphine was about 95±5% and data were corrected for this recovery.

DISCUSSION

Pretreatment with indomethacin diminished both the increase in temperature and the behavioral suppression seen after the challenge dose of morphine. This could have been due to the PG synthetase inhibitor antagonizing the initial acute effects of the opiate. However, indomethacin did not alter morphine's acute behavioral suppressant effect (this report). There is conflicting evidence about whether indomethacin alters morphine-induced hyperthermia. One report suggests that indomethacin inhibits the increase in body temperature after morphine [44], while three studies found no effect ([26,33] this report). While this is still controversial, indomethacin is probably not just affecting morphine by antagonizing the acute effects of the opiate. The effects of

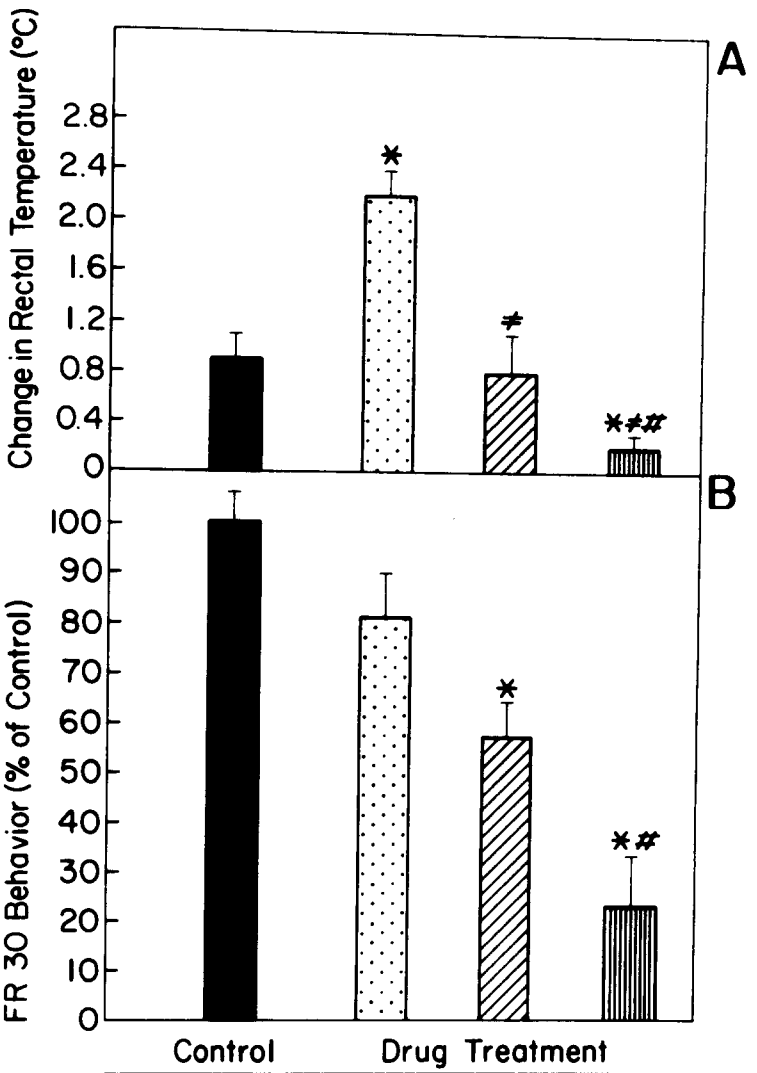

FIG. 5. Acute dependence on morphine enhanced by indomethacin. *$p<0.05$ compared with the same rats treated with vehicle, saline, and saline (2-tailed, paired Student t-test). $p<0.05$ compared with the appropriate group treated with vehicle, morphine, and saline ($\neq$) or vehicle, morphine, and naloxone (#) (analysis of variance and Duncan's new multiple range test). See Fig. 1 for an explanation of the data presentation. *Panel A*—The rats' mean temperature before the first injection was 37.2±0.2°C. *Panel B*—Their response rate was 1.81±0.11 responses/second during the control portion of the experiment. All values are mean±S.E.

indomethacin could also have resulted from it decreasing the amount of morphine in brain, thereby resulting in a smaller effect of the opiate upon adaptive processes. However, the last experiment and work by others [12] do not support this possibility. This suggests that indomethacin altered, in some other way, the adaptive processes which attend morphine administration, making the rats more tolerant to morphine. This point is also controversial since Eisenberg [12] found that indomethacin did not affect acute tolerance to morphine as evidenced by changes in plasma corticosterone levels.

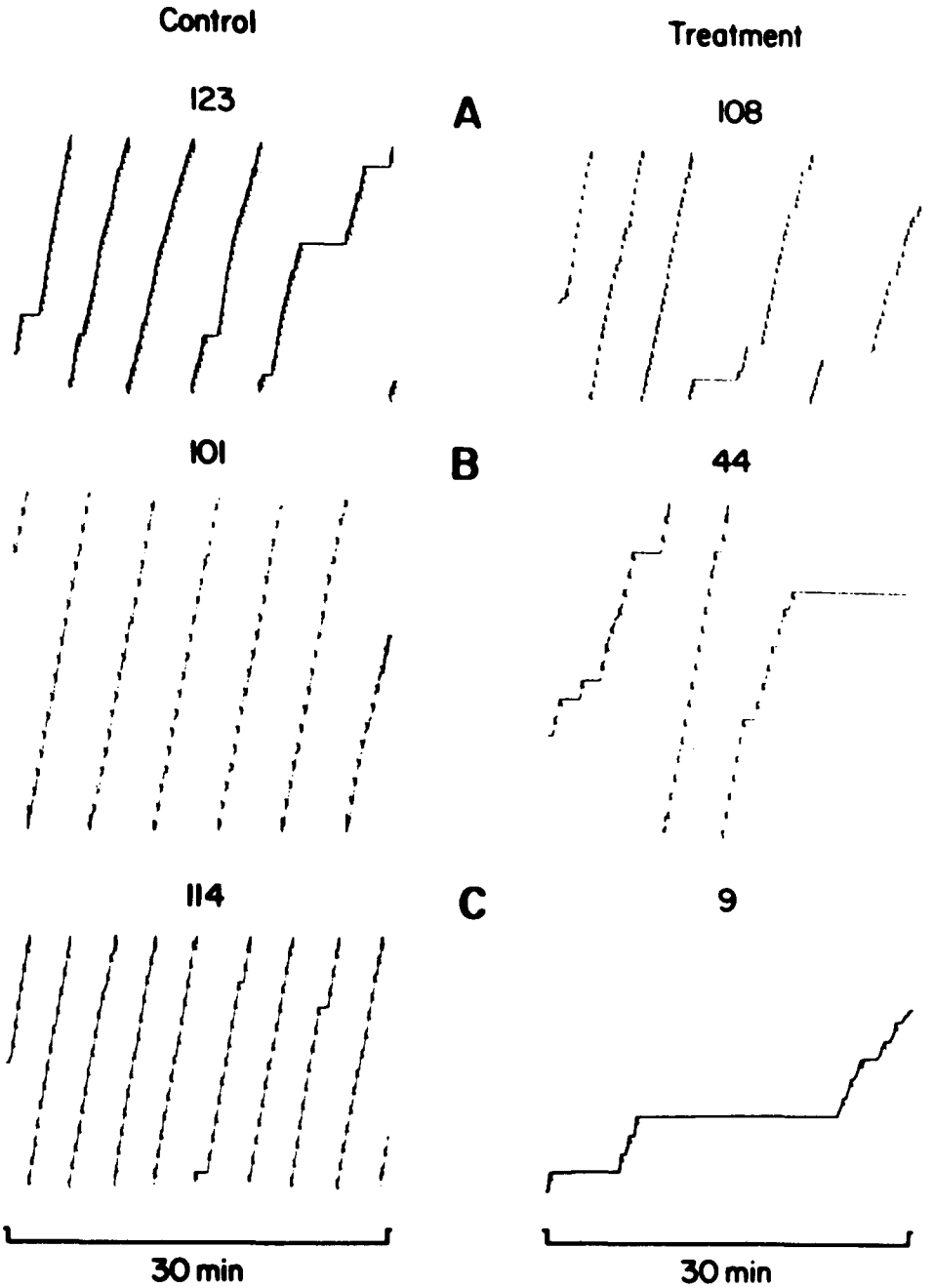

FIG. 6. Sample cumulative records from individual rats demonstrating indomethacin pretreatment potentiated naloxone-induced suppression of behavior in morphine-treated rats. Rats were injected 3 times at 3-hour intervals. Behavior shown on the cumulative records is for 30-minute periods after the third injection on control (the day before treatment) and treatment days. On the control days all rats were injected 3 times with the appropriate vehicle. On the treatment day rat 50 *(Panel A)* received vehicle, morphine, and saline; rat 52 *(Panel B)* received vehicle, morphine, and naloxone; and rat 46 *(Panel C)* received indomethacin (5 mg/kg), morphine, and naloxone. All morphine and naloxone injections were 15 and 2.5 mg/kg, respectively. See legend to Fig. 3 for additional explanations of the values above the cumulative records, slope of the recordings, and pips on the recordings.

This difference between our findings and those of Eisenberg could be due to the end points that were measured (corticosterone versus behavior and body temperature). Our suggestion that indomethacin affects the adaptive processes which occur after morphine treatment are based on measuring two variables (behavior and body temperature) during the expression of not only acute tolerance, but also acute dependence.

An effect of naloxone in morphine-treated subjects has been used to imply dependence on morphine [22]. Low doses

TABLE 2

MORPHINE-INDUCED INCREASE IN RECTAL TEMPERATURE IN RATS WAS NOT ALTERED BY INDOMETHACIN PRETREATMENT*

Pretreatment	Rectal Temperature (°C) (30 minutes after treatment) (mean ± S.E.) Saline	Morphine
Vehicle	37.3 ± 0.1	38.0 ± 0.1†
Indomethacin	37.4 ± 0.1	38.2 ± 0.2†

*Pretreatment occurred 3 hours before injection of morphine. The same 6 rats received vehicle and saline followed 3 days later by vehicle and morphine (7.5 mg/kg, IP). Six difference rats received indomethacin (5 mg/kg, IP) and saline followed 3 days later by indomethacin and morphine.

†$p<0.05$ compared with saline treatment and corresponding pretreatment (2-tailed, paired Student t-test).

TABLE 3

LACK OF EFFECT OF INDOMETHACIN ON BRAIN CONCENTRATION OF MORPHINE IN RATS

Pretreatment	Time (minutes) after morphine 20	30	50
	Morphine (ng/g brain, wet weight)		
Vehicle	181 ± 16*	241 ± 16	173 ± 23
Indomethacin (5 mg/kg, IP)	174 ± 29	271 ± 16	166 ± 14

*Mean ± S.E. (N=4 for each group).

of naloxone decreased FR behavior in a dose-related manner in morphine-dependent rats [18] when conditioned behavior was used as a measure of dependence or withdrawal. The results presented above indicate that pretreatment with indomethacin increased the effect of naloxone upon behavior in morphine-treated rats. In addition, naloxone antagonized morphine-induced hyperthermia in rats minimally tolerant to morphine, while a similar dose of naloxone further lowered body temperature in rats made more tolerant to morphine [23, 33, 40]. In the experiments reported herein, naloxone attenuated or antagonized morphine-induced hyperthermia and indomethacin pretreatment resulted in naloxone further lowering rectal temperature. These results suggest that indomethacin facilitates acute dependence on morphine.

Morphine stimulates the synthesis of PGs [4,5]. It has been hypothesized that at least one aspect of morphine tolerance or dependence involves an attenuation of morphine-induced stimulation of PG synthesis. It was reported that tolerance develops to methionine-enkephalin-induced production of PGE-like material in rat brain [34]. Therefore, a PG synthetase inhibitor might be expected to augment morphine tolerance and/or dependence. Scoto and coworkers [34] go on to suggest that opiates produce hyperthermia by increasing the synthesis of PGEs. If such is the case, pretreatment with indomethacin might be expected to attenuate morphine-induced hyperthermia by inhibiting the synthesis of PGEs. However, much controversy exists as to the role of PGEs in morphine's acute actions (e.g., [6, 7, 9, 10, 11, 16, 35, 36, 41, 43]. Our findings indicate that the adaptive processes which occur after morphine administration (i.e., tolerance and dependence) are more sensitive to changes in PGs than are the acute effects of morphine. This conclusion is supported by the findings reported in the accompanying paper where infusion, intracerebroventricularly, of doses of PGE_2 and $PGF_{2\alpha}$ which had no effect on the acute behavioral suppressant action of morphine, altered the rats dependence on morphine [28]. Increasing the ratio of $PGE_2/PGF_{2\alpha}$ in rat brain by infusing PGE_2 into the ventricles led to a greater effect of naloxone in opiate-dependent rats, while decreasing the ratio by infusing $PGF_{2\alpha}$ intracerebroventricularly led to a smaller effect of naloxone in opiate-dependent rats. Those data suggest that it is not the absolute amount of PGE in the CNS that is important during naloxone-induced withdrawal in morphine-dependent rats, but the *ratio* of PGs, perhaps PGE_2 to $PGF_{2\alpha}$; and that naloxone's effects during opiate dependence are increased by raising the $PGE_2/PGF_{2\alpha}$ ratio and decreased by lowering this ratio. Support for this suggestion comes from the finding that indomethacin decreases the amount of PGE_2 and increases the ratio of $PGE_2/PGF_{2\alpha}$ in rat brain [1,16], and this drug potentiates the effects of naloxone in opiate-dependent rats.

To summarize, indomethacin potentiated tolerance to and dependence on morphine, as measured by changes in FR 30 behavior and rectal temperature in rats. At least a partial explanation for these results is that indomethacin is inhibiting the synthesis of PGs which are important in morphine tolerance and dependence.

REFERENCES

1. Abdel-Halim, M. S., B. Sjöquist and E. Änggård. Inhibition of prostaglandin synthesis in rat brain. *Acta Pharmacol Toxicol* **43**: 266–272, 1978.
2. Babbini, M., M. Gaiardi and M. Bartoletti. Changes in fixed-interval behavior during chronic morphine treatment and morphine abstinence in rats. *Psychopharmacologia* **45**: 255–259, 1976.
3. Babbini, M., M. Gaiardi and M. Bartoletti. Changes in operant behavior as an index of withdrawal state from morphine in rats. *Psychon Sci* **29**: 142–144, 1972.
4. Bekemeier, H., A. J. Giessler and E. Vogel. Influence of MAO-inhibitors, neuroleptics, morphine, mescaline, divascan, aconitine, and pyrogens on prostaglandin biosynthesis. *Pharmacol Res Commun* **9**: 587–598, 1977.
5. Collier, H. O. J., W. J. McDonald-Gibson and S. A. Saeed. Apomorphine and morphine stimulate prostaglandin biosynthesis. *Nature* **252**: 56–58, 1974.
6. Collier, H. O. J. and A. C. Roy. Morphine-like drugs inhibit the stimulation by E prostaglandins of cyclic AMP formation by rat brain homogenate. *Nature* **248**: 24–27, 1974.

7. Collier, H. O. J. and A. C. Roy. Hypothesis: Inhibition of E prostaglandin-sensitive adenyl cyclase as the mechanism of morphine analgesia. *Prostaglandins* **7:** 361–376, 1974.
8. Cox, B., T-F. Lee and M. J. Vale. Effects of morphine and related drugs on core temperature of two strains of rat. *Eur J Pharmacol* **54:** 27–36, 1979.
9. Dismukes, K. and J. W. Daly. Accumulation of adenosine 3′,5′-monophosphate in rat brain slices: Effects of prostaglandins. *Life Sci* **17:** 199–209, 1975.
10. Ehrenpreis, S., J. Greenberg and S. Belman. Prostaglandins reverse inhibition of electrically-induced contractions of guinea pig ileum by morphine, indomethacin, and acetylsalicylic acid. *Nature New Biol* **245:** 280–282, 1973.
11. Ehrenpreis, S., J. Greenberg and J. E. Comaty. Block of electrically induced contractions of guinea pig longitudinal muscle by prostaglandin synthetase and receptor inhibitors. *Eur J Pharmacol* **39:** 331–340, 1976.
12. Eisenberg, R. M. Short-term tolerance to morphine: Effects of indomethacin. *Life Sci* **30:** 1399–1405, 1982.
13. Feldberg, W. and P. N. Saxena. Fever produced by prostaglandin E_1. *J Physiol (Lond)* **217:** 547–556, 1971.
14. Feldberg, W. and P. N. Saxena. Prostaglandins, endotoxin and lipid A on body temperature in rats. *J Physiol (Lond)* **249:** 601–615, 1975.
15. Fernandes, M., S. Kluwe and H. Coper. The development of tolerance to morphine in the rat. *Psychopharmacology (Berlin)* **54:** 197–201, 1977.
16. Ferri, S., A. Santagostina, P. C. Braga and I. Galatulas. Decreased antinociceptive effect of morphine in rats treated intraventricularly with prostaglandin E_1. *Psychopharmacologia* **39:** 231–235, 1974.
17. Fitzpatrick, F. A. and M. A. Wynaldo. *In vivo* suppression of prostaglandin biosynthesis by non-steroidal anti-inflammatory agents. *Prostaglandins* **12:** 1037–1051, 1976.
18. Gellert, V. F. and S. B. Sparber. A comparison of the effects of naloxone upon body weight loss and suppression of fixed-ratio operant behavior in morphine-dependent rats. *J Pharmacol Exp Ther* **201:** 44–54, 1977.
19. Goldberg, S. R. Nalorphine: Conditioning of drug effects on operant performance. In: *Stimulus Properties of Drugs*, edited by T. Thompson and R. Pickens. New York: Appleton-Century-Crofts, 1971.
20. Gunne, L.-M. The temperature response in rats during acute and chronic morphine administration. A study of morphine tolerance. *Arch Int Pharmacodyn* **129:** 416–428, 1960.
21. Hermann, J. B. The pyretic action on rats of small doses of morphine. *J Pharmacol Exp Ther* **76:** 309–318, 1942.
22. Jasinski, D. R., W. R. Martin and C. A. Haertzen. The human pharmacology and abuse potential of naloxone. *J Pharmacol Exp Ther* **157:** 420–426, 1967.
23. Linseman, M. A. Naloxone-precipitated withdrawal as a function of the morphine-naloxone interval. *Psychopharmacology (Berlin)* **54:** 159–164, 1977.
24. Martin, G. E., A. T. Pryzbylik and N. H. Spector. Restraint alters the effects of morphine and heroin on core temperature in the rat. *Pharmacol Biochem Behav* **7:** 463–469, 1977.
25. Meyer, D. R. and S. B. Sparber. Evidence of possible opiate dependence during the behavioral depressant action of a single dose of morphine. *Life Sci* **21:** 1087–1093, 1977.
26. Milton, A. S. and S. Wendlandt. A possible role for prostaglandin E_1 as a modulator for temperature regulation in the central nervous system of the cat. *J Physiol (Lond)* **207:** 76–77P, 1970.
27. Mucha, R. F., H. Kalant and M. A. Linseman. Quantitative relationships among measures of morphine tolerance and physical dependence in the rat. *Pharmacol Biochem Behav* **10:** 397–405, 1979.
28. Nielsen, J. A. and S. B. Sparber. Central administration of prostaglandin E_2 facilitates while $F_{2\alpha}$ attenuates acute dependence upon morphine in rats. *Pharmacol Biochem Behav* **22:** 933–939, 1985.
29. Oka, T., M. Nozaki and E. Hosoya. Effects of p-chlorophenylalanine and cholinergic antagonists on body temperature changes induced by the administration of morphine to nontolerant and morphine-tolerant rats. *J Pharmacol Exp Ther* **180:** 136–143, 1972.
30. Potts, W. J. and P. F. East. Effects of prostaglandin E_2 on the body temperature of conscious rats and cats. *Arch Int Pharmacodyn Ther* **197:** 31–36, 1972.
31. Ramírez-Solares, R., M. Lujan and R. Rodriguez. Evidences for involvement of prostaglandins of the E series in morphine physical dependence in the isolated ileum of the guinea pig. *Proc West Pharmacol Soc* **26:** 345–350, 1983.
32. Rhodus, D. N., T. F. Elsmore and F. J. Manning. Morphine and heroin effects on multiple fixed-interval schedule performance in rats. *Psychopharmacologia* **40:** 147–155, 1974.
33. Rudy, T. A. and T. L. Yaksh. Hyperthermic effects of morphine: Set point manipulation by a direct spinal action. *Br J Pharmacol* **61:** 91–96, 1977.
34. Scoto, G. M., C. Spadaro, S. Spampinato, R. Arrigo-Reina and S. Ferri. Prostaglandins in the brain of rats given, acutely, and chronically, a hyperthermic dose of met-enkephalin. *Psychopharmacology (Berlin)* **60:** 217–219, 1979.
35. Sharma, S. K., M. Nirenberg and W. A. Klee. Morphine receptors as regulators of adenylate cyclase activity. *Proc Natl Acad Sci USA* **72:** 590–594, 1975.
36. Sparber, S. B., V. F. Gellert, L. Lichtblau and R. Eisenberg. The use of operant behavior methods to study aggression and effects of acute and chronic morphine administration in rats. In: *Factors Affecting the Action of Narcotics*, edited by M. W. Adler, L. Manara and R. Samanin. New York: Raven Press, 1978.
37. Steel, R. G. D. and J. H. Torrie. *Principles and Procedures of Statistics: A Biomedical Approach*. New York: McGraw-Hill, 1980.
38. Tell, G. P., G. W. Pasternak and P. Cuatrecasas. Brain and caudate nucleus adenylate cyclase: Effects of dopamine, GTP, E prostaglandins and morphine. *FEBS Lett* **51:** 242–245, 1975.
39. Thompson, T. and C. R. Schuster. Morphine self-administration, food-reinforced and avoidance behaviors in Rhesus monkeys. *Psychopharmacologia* **5:** 87–94, 1964.
40. Thornhill, J. A., M. Hirst and C. W. Gowdey. Changes in the hyperthermic responses of rats to daily injections of morphine and the antagonism of the acute response by naloxone. *Can J Physiol Pharmacol* **56:** 483–489, 1978.
41. Traber, J., K. Fischer, S. Latzin and B. Hamprecht. Morphine antagonises action of prostaglandin in neuroblastoma and neuroblastoma times glioma hybrid cells. *Nature* **253:** 120–122, 1975.
42. Tulunay, F. C., I. Yano and A. E. Takemori. The effect of biogenic amine modifiers on morphine analgesia and its antagonism by naloxone. *Eur J Pharmacol* **35:** 285–292, 1976.
43. Van Inwegen, R. G., S. J. Strada and G. A. Robison. Effects of prostaglandins and morphine on brain adenylyl cyclase. *Life Sci* **16:** 1875–1876, 1975.
44. Wallenstein, M. C. Effect of prostaglandin synthetase inhibitors on non-analgesic actions of morphine. *Eur J Pharmacol* **90:** 65–73, 1983.