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Tolerance and Evidence of Physical Dependence to Daily Codeine Injections in the Rat

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THORNHILL, J. A., M. HIRST AND C. W. GOWDEY. *Tolerance and evidence of physical dependence to daily codeine injections in the rat.* PHARMAC. BIOCHEM. BEHAV. 9(4) 433–438, 1978.—Core temperatures, measured by telemetry, and acquisition of food pellets on a continuous reinforcement schedule were recorded every 30 min in unrestrained male rats given saline for 5 days before and 5 days after 10 daily SC injections of codeine phosphate (200 mg/kg) at 08:00 hr. After the first codeine injection rats were immobile, slightly catatonic, breathed shallowly and had elevated core temperatures, loss of body weight and inhibition of feeding activity. As injections of codeine were repeated, the initial depressant signs decreased and the period of inhibited feeding was replaced by prolonged (>8 hr) post-injection bouts of feeding activity (stimulated feeding) during daylight hours. Core temperatures remained elevated during this phase of drug-induced feeding activity. Mean body weight and 24-hr food intake remained below control levels over the 10-day codeine period as diurnal feeding patterns became reversed. On the first withdrawal day core temperatures declined and feeding patterns changed from those responses on the last codeine day as the rats lost body weight and were hyperirritable. As withdrawal continued core temperature and feeding patterns began to resemble those of the saline control period, body weights increased and hyperirritability subsided. In this study, tolerance and evidence of physical dependence to daily injections of codeine could be demonstrated in rats by continuous monitoring of their diurnal feeding and temperature responses.

Daily SC codeine injections Core temperature Hyperthermia Feeding patterns Stimulated feeding
Tolerance Diurnal feeding disruptions Withdrawal Body weight loss Hyperirritability

IN THIS laboratory repeated daily injections of heroin to rats, even during daylight hours, were found to alter feeding behavior [26] and core temperature responses [25] in such a way that postinjection bouts of vigorous feeding activity (stimulated feeding) and increased hyperthermia were more prominent as the injections continued. These changes in temperature and feeding were correlated temporally and were considered [25] to indicate tolerance development. On withdrawal from opiates, decreases in core temperature have been observed in physically-dependent rats [15, 16, 25, 32] along with characteristic reductions in body weight [1, 7, 10, 17, 24, 25], dose-dependent disruptions in feeding [25,26], increased irritability [23,25], and other behavioral signs [2, 13, 15–26, 29–31]. It was considered important to determine whether repeated daily injections of a narcotic agent such as codeine, with less analgesic activity [5] and little dependence-producing liability [20], would evoke patterns of change in core temperature and feeding activity comparable to those after morphine or heroin.

METHOD

Core temperatures were measured in male Sprague-Dawley rats (450–550 g) from small, Silastic covered, precalibrated thermistor pellets (Temtron Electronics, London, Canada), surgically implanted in the abdominal cavity with sterile precautions. The outputs from these thermistors were recorded telemetrically with broad-band AM radio receivers and each half hour the core temperature of each rat was printed out via a data logger. At least 1 week after the surgery the rats were placed individually in operant conditioning chambers in a controlled environment (temperature ranging between 24–27°C, humidity ~40%) and taught to obtain their total food intake by bar pressing for 45 mg food pellets on a continuous reinforcement schedule by a method previously described [26]. The experimental room was maintained on a 12-hr on-off lighting schedule (lights on from 08:00 to 20:00 hr daily). The body weight of each rat was measured every morning prior to drug administration and these pre-injection weights were used to calculated the daily

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drug doses. The feeding activity of each rat was monitored continually and recorded every 30 min throughout the 20-day experiment with a second data logger.

After allowing 2–3 days for the diurnal pattern of food intake of each rat to stabilize, an injection of 0.9% w/v saline (0.25 ml/100 g body weight) was given subcutaneously (SC) to 5 rats at 08:00 hr (beginning of light period) on each of 5 days. Following this control period the group received daily SC injections of codeine phosphate (200 mg/kg, British Drug Houses) dissolved in similar volumes of saline at 08:00 hr for 10 days. Withdrawal was induced in the group by substituting saline for the previous codeine injections. The abstinence period was continued for 5 days.

A family of temperature curves (1, 2, 4 and 8-hr postinjection) of each of the 20 experimental days was plotted, using constant coordinates, for each rat. Using a common baseline of 36.0°C the 1, 2, 4 and 8-hr areas of each temperature curve on each experimental day (during saline control, codeine, and saline withdrawal periods) were measured twice for every rat by a planimeter and averaged. The 1, 2, 4 and 8-hr areas (Celsius degrees × hours) are referred to as core temperature indices (CTI-1 hr, CTI-2 hr, CTI-4 hr, CTI-8 hr, respectively). These were measured and the means derived for each experimental day. The CTI's at several times after administration (1, 2, 4 or 8-hr) on various days were analyzed statistically by the Student's t test for paired data. Mean food pellet consumption (4 and 8-hr postinjection amounts as well as 12-hr light and dark totals, and for the whole 24-hr period) on various treatment days were compared using Student's t test for paired data. Mean changes in body weight between various experimental days were also evaluated statistically by this method. Unless otherwise stated a $p<0.05$ was considered to reflect a significant change. The degree of irritability of each rat over the 5-day period of withdrawal was assessed and scored according to criteria described by Teiger [23].

Comparison of the obtained measurements of core temperature by the telemetric method with those taken periodically each day by a rectal probe (Yellow Springs Telethermometer) showed good agreement (within 0.2°C) over the entire 20-day experiment. The implanted transmitter capsule caused no hindrance to the rats' movements after postoperative recovery. Post mortem histological examinations showed encapsulation of the pellet by well differentiated fibrous tissue, but no evidence of a purulent inflammatory reaction.

RESULTS

Figure 1 shows the mean responses of core temperature and feeding activity in the group of rats over an 8-hr postinjection period on several experimental days following injections of saline or codeine phosphate (200 mg/kg). It can be seen that a hyperthermic response occurred after the first codeine injection which peaked after 3 hr. Significant increases in CTI at 4 and 8 hr (Table 1) were found when these were compared to those derived from the previous saline injection. Feeding was abolished completely over the entire 8-hr period on this day and for much of this time the rats were immobile, catatonic, and showed shallow breathing and exophthalmos. These signs had virtually disappeared by the tenth codeine injection, on which day a mild hyperthermia (~38.0°C) was observed that persisted over the whole 8-hr period. Table 1 shows that over the 10 days of codeine injections core temperature indices (CTI-1, 2, 4 or 8-hr) did not change statistically from those after the first codeine injection. Many CTI-4 hr and all CTI-8 hr, however, were significantly higher than those obtained for the last saline control injection. Although not shown in Fig. 1, feeding activity in these rats occurred progressively sooner after each codeine injection. By the tenth day of codeine administration feeding activity occurred within the first 30 min and food intake increased significantly over the first 4-hr postinjection beyond that found after the first codeine or the fifth saline injections.

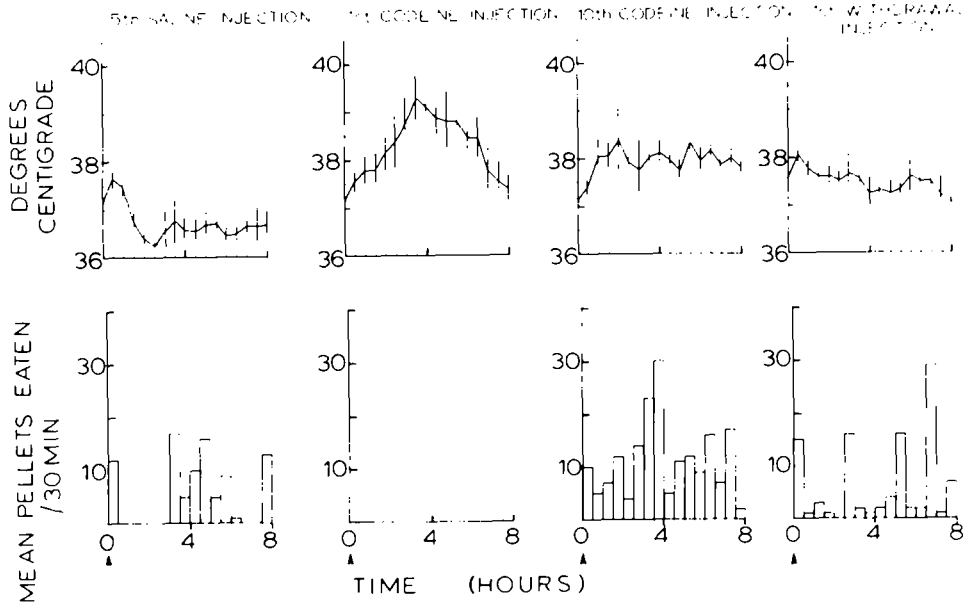

FIG. 1. Mean core temperatures (± SEM) and feeding activity (mean pellets consumed/30 min) are shown over an 8-hr post-injection period on several experimental days for the group of rats (n = 5) given saline or 200 mg/kg codeine phosphate daily at 08:00 hr. Arrows denote time of drug injections.

TABLE 1

MEAN CORE TEMPERATURE INDICES, CTI (°C×hr) ± SEM FOR RATS (N=5) TREATED WITH CODEINE PHOSPHATE (200 MG/KG, SC)

Day	C.T.I. (1 hr)	(2 hr)	(4 hr)	(8 hr)
1st Saline	1.83 ± 0.156	3.69 ± 0.148	5.58 ± 0.438	8.25 ± 0.887
2nd Saline	1.51 ± 0.119	2.84 ± 0.319	4.48 ± 0.643	7.14 ± 0.758
3rd Saline	1.91 ± 0.116	3.31 ± 0.221	4.53 ± 0.422	7.32 ± 1.008
4th Saline	1.78 ± 0.242	3.00 ± 0.485	4.96 ± 0.843	7.41 ± 1.252
5th Saline	1.71 ± 0.234	2.75 ± 0.433	4.29 ± 0.867	7.15 ± 1.558
1st Codeine	1.63 ± 0.176	3.53 ± 0.319	8.96 ± 1.020*	18.72 ± 2.246*
2nd Codeine	1.86 ± 0.152	3.49 ± 0.384	7.47 ± 1.090*	16.76 ± 1.580*
3rd Codeine	1.54 ± 0.084	2.99 ± 0.131	6.28 ± 0.554	14.89 ± 0.952*
4th Codeine	1.67 ± 0.086	3.21 ± 0.270	7.34 ± 0.433*	17.12 ± 0.638*
5th Codeine	1.39 ± 0.126	3.37 ± 0.480	7.16 ± 1.088	14.88 ± 1.703*
6th Codeine	1.87 ± 0.119	3.62 ± 0.162	7.33 ± 0.586*	15.24 ± 0.917*
7th Codeine	2.10 ± 0.263	4.45 ± 0.561	8.85 ± 1.063*	19.53 ± 1.325*
8th Codeine	1.61 ± 0.086	3.56 ± 0.437	6.94 ± 1.129	15.63 ± 1.543
9th Codeine	1.97 ± 0.160	3.91 ± 0.248	7.31 ± 0.867	14.80 ± 1.283
10th Codeine	1.44 ± 0.096	3.56 ± 0.330	7.89 ± 0.980*	15.48 ± 0.937
1st Withdrawal	1.98 ± 0.254†	3.40 ± 0.209	6.40 ± 0.620	13.05 ± 1.901
2nd Withdrawal	1.43 ± 0.152	3.04 ± 0.276	5.38 ± 0.524	10.84 ± 0.975
3rd Withdrawal	1.53 ± 0.196	2.86 ± 0.357	5.29 ± 0.505	9.59 ± 0.847
4th Withdrawal	1.53 ± 0.146‡	2.93 ± 0.222‡	5.19 ± 0.441‡	9.14 ± 0.994
5th Withdrawal	1.21 ± 0.107‡	2.28 ± 0.207‡	4.01 ± 0.413‡	7.78 ± 0.923‡

*Significant change ($p<0.05$) from last saline control day.
†Significant change ($p<0.05$) from 10th codeine day.
‡Significant change ($p<0.05$) from 1st withdrawal day.

On the first withdrawal day the mean core temperature responses over 8-hr lay between those of the final codeine injection and fifth saline control injection and most CTIs did not differ significantly from those of the latter experimental days. On the first withdrawal day food intakes over the first 4 and 8-hr were decreased significantly from the amounts eaten during the same time periods after the previous, tenth codeine injection. By the fifth day of withdrawal all CTI's were significantly decreased from those on the last codeine treatment day and similar to those of the fifth saline control day (Table 1). Postinjection bouts of vigorous feeding activity did not occur during the 5 days of withdrawal injections. Feeding during the light period was intermittent, a pattern more resembling that observed after the fifth saline control injection.

The diurnal pattern of food intake on several experimental days for the group of codeine-treated rats is shown in Table 2. After the first codeine injection the mean 24-hr food intake decreased significantly from that taken on the previous saline control day. This decrease in total food intake occurred from reduced feeding during both the 12-hr light ($p<0.01$) and dark periods ($p<0.01$). As codeine treatment continued, the amounts eaten during the 12-hr light periods increased to the point that it represented over 50% of the total daily food intake, a reversal in the 24-hr food aquisition pattern that was seen during the saline control period. Table 2 shows that by the fifth codeine day these changes were significant and continued to the final codeine day. On the first day of withdrawal, total food intake tended to decline, but this change was not significant. By the fifth withdrawal day the percentages of daily food intake consumed during the light and dark periods more closely resembled those of the saline control period, but the total food consumption was greater than that taken on the tenth codeine and first withdrawal days.

Figure 2 shows changes in mean body weight, as a percentage of the weight on the last saline control day, for the rats during the codeine treatment and subsequent withdrawal period. During the codeine treatment period the rats showed a progressive decline in body weight and, from the third to the tenth day, body weights were significantly below ($p<0.01$) those on the first codeine day. Additional significant decreases occurred in these rats by the end of the first withdrawal day when compared to body weights on the previous tenth codeine day. The abrupt changes in body weight seen on the first saline withdrawal day were accompanied by signs of extreme irritability on being touched or grasped, as shown by the irritability scores on the figures; in addition, tremors, piloerection, ptosis, increased urination and defecation, and hair loss was noted. These signs of hyperirritability persisted until the third day of withdrawal at which time body weights began to increase. By the fifth withdrawal day body weights were rising steadily.

DISCUSSION

The hyperthermic response following the first injection of codeine phosphate (200 mg/kg) qualitatively resembled the temperature responses reported after a single injection of a low dose of morphine [3, 6, 8, 9, 18, 22, 27, 33], heroin [25] or codeine [6]. The hyperthermia induced by codeine was less intense but more prolonged than that after morphine [8,27] or

TABLE 2
24-HOUR PATTERN OF FOOD INTAKE

Group	Experimental Day	Mean 12-Hr Light Period Food Intake (G ± SD)	Mean 24-Hr Food Intake (G ± SD)	Light Period Intake % Total 24-Hr Intake
	(a) 5th Saline	6.59 ± 1.14	24.21 ± 4.23	27.2
200 mg/kg	(b) 1st Codeine	0.47 ± 0.51*	3.80 ± 2.95*	12.4
Codeine	(c) 5th Codeine	10.49 ± 4.23†	17.10 ± 4.89†	61.6*
Phosphate	(d) 10th Codeine	9.51 ± 3.72‡	16.20 ± 5.38‡	58.7*
(n 5)	(e) 1st Withdrawal	5.66 ± 3.43	14.28 ± 5.20	39.6
	(f) 5th Withdrawal	11.35 ± 5.81	30.38 ± 7.48‡§	37.4

*Significant change ($p < 0.05$) from last saline control day
†Significant change ($p < 0.05$) from 1st codeine day
‡Significant change ($p < 0.05$) from 1st withdrawal day
§Significant change ($p < 0.05$) from 10th codeine day

heroin [25]. With repeated administrations of codeine in this study the peak hyperthermia did not progressively increase in intensity as it was reported to do in restrained rats [6] with daily injections of 75 mg/kg codeine phosphate or after injections of other opiates [6, 8, 25, 27]. Temperature responses on both the first and fifth days of withdrawal from codeine resembled those previously observed in rats withdrawn from repeated injections of heroin [25].

The pattern of change in feeding activity from inhibition after the initial injection of codeine to pronounced stimulated feeding with repeated injections was taken to indicate that tolerance had developed. Other signs of depressant narcotic analgesic activity decreased concomitantly over the period of codeine treatment. Similar alterations have been shown to occur during chronic treatments with other opiates [14, 25–27]. As with the temperature responses, the duration of stimulated feeding was longer with codeine. Because of this prolonged, codeine-induced feeding in the daylight period,

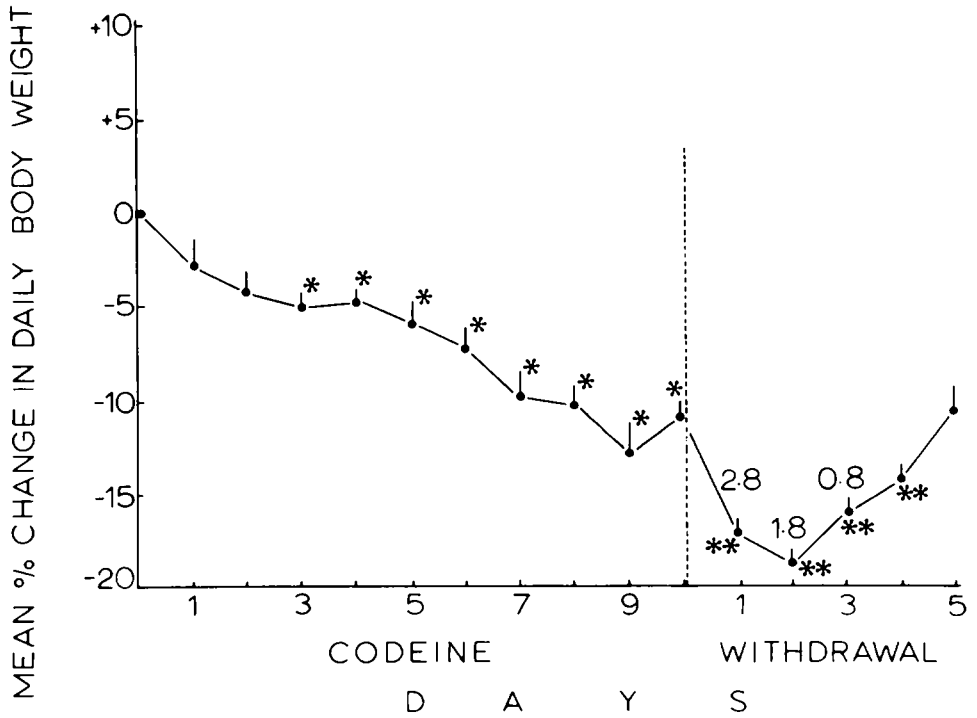

FIG. 2. Daily changes in mean body weight (mean % change from the last saline control weight ± SEM) are shown over the 10 days of codeine treatment and subsequent 5-day withdrawal period for the group of rats given saline or codeine phosphate (200 mg/kg) daily. Mean daily irritability scores are shown for the group during the withdrawal period. Mean body weight ± SEM in g on the last saline control day was 514.0 ± 56.95. *denotes significant change ($p < 0.05$) in mean body weight of that group from their weights at the end of the last saline control day. **denotes significant change ($p < 0.05$) in mean body weight of that group from their weights at the end of the 10th codeine day.

the diurnal pattern of food intake became reversed from that in the control period; a result observed earlier with heroin [25].

The persistent, drug-induced hyperthermia and coincident stimulated feeding seen after repeated injections of codeine suggests that the drug has a long duration of action in the rat. These prolonged responses could be related to a slow metabolism to morphine [4, 5, 11, 12, 28, 34], for morphine has been shown to produce similar qualitative temperature and feeding responses in the rat [6, 8, 27].

Codeine has been generally considered to induce little physical dependence [19,21] and yet the results from careful monitoring of diurnal patterns of body temperature and food intake, irritability and weight loss in the withdrawal component of this study indicate definite signs of physical dependence in rats given single, daily injections of the drug. Similar changes in body weight and irritability scores after codeine were reported from a study [23] in which the drug was constantly infused in comparable daily doses through intraperitoneal cannulae and the rats withdrawn abruptly by substituting dextrose for the codeine solution. Both the intensity and duration of physical dependence as measured in the present experiments on withdrawal from codeine phosphate (200 mg/kg/day) by saline were greater than that found in earlier experiments on saline withdrawal from daily injections of 20 mg/kg heroin hydrochloride [25]. The reason for the observed degree of physical dependence following withdrawal of codeine may be related to its long duration of action.

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