*Pharmacology Biochemistry & Behavior*, Vol. 18, pp. 495–499, 1983. 

# Restraint Alters the Thermic Response to Morphine by Postural Interference

J. N. McDOUGAL,[1] P. R. MARQUES AND T. F. BURKS

*Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724*

Received 27 July 1981

McDOUGAL, J. N., P. R. MARQUES AND T. F. BURKS. *Restraint alters the thermic response to morphine by postural interference.* PHARMACOL BIOCHEM BEHAV 18(4) 495–499, 1983.—The effects of morphine on body temperature have been shown to be altered by restraint. The purpose of this study was to determine how the type of restraint alters body temperature measurements and whether restraint alters the effects of morphine on body temperature by interfering with the ability of the rats to adjust their posture. The thermic effects of 5 doses of morphine (3.8 to 45 mg/kg) were compared in two types of restraint and confinement to a 13×20×20 cm pan without restraint. In unrestrained rats, morphine caused predominantly hyperthermia, but with restraint morphine caused hyperthermia at low doses and hypothermia at higher doses. Morphine hypothermia was greater in rats restrained in a wire-mesh restrainer which prevented heat and humidity build-up than in the commonly used plastic restrainer. In the unrestrained rats, morphine treatment was associated with a posture characterized by exophthalamos, immobility, a hunched position and increased muscle tone. Restrained rats could not assume a compact posture. These results suggest that restraint alters the thermic effect of morphine mainly by interfering with postural mechanisms which reduce heat loss.

Body temperature Morphine Restraint Stress Thermoregulation

THE effect of morphine on body temperature depends on the dose and route of administration [4,12]. In the rat, small doses of morphine (1–10 mg/kg) cause hyperthermia and larger doses cause hypothermia. Morphine-induced hyperthermia is a result of slightly increased heat production and decreased heat loss, accompanied by increased activity of sympathetic outflow [10]. Morphine-induced hypothermia is due to decreased heat production and increased heat loss [19] and/or loss of thermoregulatory control [5]. Most studies which have investigated the effects of morphine on body temperature have utilized restrained rats. Rectal temperature is usually measured in rats which are restrained in plastic restrainers [13,20], or wire-mesh restrainers [17,21]. Some investigators avoid the need for restraint by inserting a rectal probe into the rat each time a temperature measurement is desired [10,18].

Recently, restraint has been shown to alter the thermoregulatory response to morphine [14, 16, 23]. In non-restrained rats morphine causes only hyperthermia whether injected systemically [8,16] or centrally [14,23]. Explanations for the different responses to morphine in restrained and free-moving rats have focused on restraint as a stress which is presumed to alter the morphine response, possibly because of endorphin release [8,22]. Other factors which could explain the differences between morphine responses in restrained and non-restrained rats are interference with postural thermoregulation and piloerection as well as the insulating effect of a restraint device. The purpose of this study was to determine contributions of behavioral thermoregulation and insulation on the thermic dose-response effect of morphine by comparing 3 different types of restraint (two restrictive restraints and confinement to a 13×20×20 cm mouse pan).

## METHOD

One hundred and forty-seven male Sprague-Dawley rats (Hilltop Laboratory Animals Inc. and Division of Animal Resources, University of Arizona) weighing 200 to 300 g were used in these studies. They were housed in the animal facility in groups of 5 to 7 in plastic cages, fed laboratory ration and water ad lib and maintained on a twelve hour light/dark cycle with the lights on at 0600. No rats were used in more than one experiment.

Thermoregulatory responses to drug treatments were quantified during a 1 hr baseline period and then 4 hr subsequent to drug administration. All injections were given between 1030 and 1200 to avoid diurnal variation. Injections of morphine sulfate (Merck Chemical Co.; Rahway, NJ) dissolved in bacteriostatic sodium chloride solution (Elkins-Sinn, Inc.; Cherry Hill, NJ) or sodium chloride solution alone were given subcutaneously at the back of the neck in a volume of 1 ml/kg body weight. The doses of morphine (3.8–45 mg/kg) were chosen to cover the whole range of thermoregulatory responses. Rectal temperatures were measured with Yellow Spring Instrument thermistors (YSI #401) inserted 6 cm into the rectum and taped to the base of the tail. Temperatures were recorded at 15 min intervals with a Tele-thermometer (YSI #46TUC).

[1]Requests for reprints should be addressed to Dr. James N. McDougal, Toxic Hazards Division, Air Force Aerospace Medical Research Laboratory, Wright-Paterson Air Force Base, OH 45433.

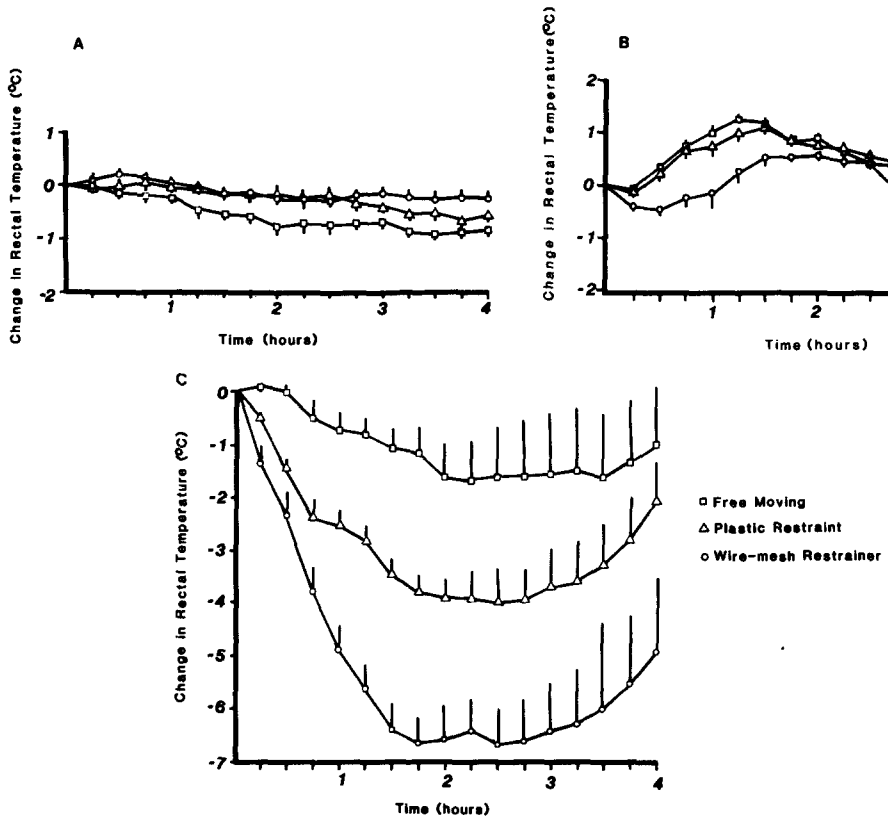


FIG. 1. Rectal temperature responses to saline and representative hyper- and hypothermic doses of morphine. Rats were restrained in wire mesh restrainers (circles), plastic restrainers (triangles) or confined to a mouse pan (squares). Two to three hours after being placed in restraint or confinement, saline (A), 3.8 mg/kg morphine (B) and 30 mg/kg morphine (C) were injected subcutaneously and rectal temperature was monitored every 15 min for 4 hr after injection. Responses are expressed as °C±SE change from baseline (mean of 4 temperature measurements prior to injection). The thermic response indices for these doses are included in Tables 1 and 2.

Rectal temperatures of 5–7 rats in each of 3 restraint conditions were monitored during each experiment. To allow one group freedom of movement and behavioral thermoregulation during temperature measurement, rats were confined in a metal mouse pan with lid (13×20×30 cm) which had an absorbent pad taped inside the bottom of the pan. The other rats were more tightly restrained in rigid plastic restrainers (Scientific Products #A4570-2, Tempe, AZ) and a locally fabricated wire-mesh restraint rack of our own design. The wire-mesh rack, which avoided heat and humidity accumulation characteristic of the plastic restrainers, was constructed on a base of expanded galvanized mesh which was elevated from the laboratory bench with 9 cm legs. This base supported ten adjustable clamshell type restrainers, made of galvanized hardware cloth, which were gently folded over the rats and fastened. The rat's tail and the thermistor probe lead wire protruded through a small opening in the aluminum back which was fastened to the base with bolts. The interior length of both the plastic and the wire-mesh restrainers was 15 cm.

Deviations of rectal temperature from baseline after injection were determined at each 15 min time point by subtracting each rat's rectal temperature from the baseline (defined as the mean of the four 15 min temperatures prior to drug injection). Thermal response index (TRI) was calculated for each rat [6] during a 4 hr period after injection. TRI is a measurement of the area of the deviation of rectal temperature from baseline and one TRI unit (deg·hr) is equivalent to a 1°C change from baseline of 1 hr duration. Both a negative TRI from rectal temperatures less than baseline and a positive TRI from rectal temperatures greater than baseline were calculated for each rat using a Texas Instruments programable calculator (TI-59). Mean TRIs were determined for each measurement condition including saline. Statistical differences were determined using analysis of variance or Dunnett's *t*-test with the alpha level set at 0.05 [24].

## RESULTS

When rats were first placed into the wire-mesh or rigid plastic restrainers, they struggled slightly and occasionally vocalized or rolled or tried to chew the restrainer during the first 30 minutes of restraint. After approximately an hour they lay quietly or infrequently groomed. Rats confined in the mouse pans initially explored the pan and then occasionally groomed or explored the rectal probe. Rats in the mouse pans were continuously observed in order to keep them from becoming tangled in or chewing the thermistor leads. After morphine injections, rats in either type of restraint remained still but occasionally pressed their nostrils against the forward wall of the restrainer until there was danger of suffocation. When this occurred a gentle tug on the tail was usually sufficient to cause the rat to shift position. In

TABLE 1

HYPERTHERMIC THERMAL RESPONSE INDICES INDUCED BY MORPHINE DURING DIFFERENT TYPES OF RESTRAINT

| Drug | Thermal Response Index (deg·hr) Wire-mesh Restraint | Plastic Restraint | Confined | $p$ |
|---|---|---|---|---|
| Saline | 0.31 ± 0.11 | 0.18 ± 0.12 | 0.04 ± 0.02 | NS |
| 3.8 mg/kg morphine | 1.06 ± 0.25* | 1.82 ± 0.23* | 1.86 ± 0.24* | NS |
| 7.5 mg/kg morphine | 1.27 ± 0.25* | 2.26 ± 0.37* | 1.70 ± 0.05* | NS |
| 15 mg/kg morphine | 0.66 ± 0.22 | 3.51 ± 0.86* | 2.41 ± 0.58* | 0.05 |
| 30 mg/kg morphine | 0.03 ± 0.03 | 0 ± 0 | 1.18 ± 0.54* | NS |
| 45 mg/kg morphine | 0 ± 0 | 0.11 ± 0.11 | 0.41 ± 0.28 | NS |

*Different from saline response in the same type of restraint, $p<0.05$.

Rectal temperatures were measured four hours at 15 minute intervals. Results are expressed as positive deviation from baseline ± S.E. with units of deg·hr. Differences in response due to type of restraint were tested with analysis of variance. Morphine responses were tested for difference from saline in the same type of restraint with Dunnett's $t$-test.

TABLE 2

HYPOTHERMIC THERMAL RESPONSE INDICES INDUCED BY MORPHINE DURING DIFFERENT TYPES OF RESTRAINT

| Drug | Thermal Response Index (deg·hr) Wire-mesh Restraint | Plastic Restraint | Confined | $p$ |
|---|---|---|---|---|
| Saline | −0.91 ± 0.23 | −1.45 ± 0.36 | −2.63 ± 0.37 | 0.01 |
| 3.8 mg/kg morphine | −0.58 ± 0.12 | −0.37 ± 0.11 | −0.88 ± 0.22 | NS |
| 7.5 mg/kg morphine | −1.58 ± 0.34 | −0.35 ± 0.15 | −1.11 ± 0.12 | 0.01 |
| 15 mg/kg morphine | −4.51 ± 1.16 | −0.56 ± 0.31 | −1.08 ± 0.47 | 0.01 |
| 30 mg/kg morphine | −21.70 ± 2.80* | −12.10 ± 1.60* | −5.76 ± 2.60 | 0.01 |
| 45 mg/kg morphine | −20.29 ± 0.68* | −9.04 ± 1.01* | −3.28 ± 0.87 | 0.01 |

*Different from saline response in the same type of restraint, $p<0.05$.

Rectal temperatures were measured four hours at 15 minute intervals. Results are expressed as negative deviation from baseline ± S.E. with units of deg·hr. Differences in response due to type of restraint were tested with analysis of variance. Morphine responses were tested for difference from saline in the same type of restraint with Dunnett's $t$-test.

unrestrained rats, within 15 min of injection, morphine caused a behavioral response characterized by immobility, a hunched posture and increased muscle tone. The duration of the posture increased with dose.

Baseline temperatures of the rats differed according to the method of restraint (analysis of variance, $p<0.05$). Basal temperatures of the rats in the wire-mesh (37.6±0.1°C) were lower than basal temperatures of the rats in the rigid plastic restrainers (38.1±0.1°C) and the rats in the mouse pans (38.3±0.1°C). The difference in baseline temperatures due to type of restraint was very small when compared to the differences in response to morphine due to type of restraint. Temperatures on the inside surface of one of each type of restrainer were measured during an experiment at an ambient temperature of between 20 and 21°C in which 30 mg/kg of morphine sulfate was injected. The mean temperatures during the hour prior to injection were 20.3±0.2°C in the wire-mesh restrainer, 21.0±0.1°C in the mouse pan and 25.0±0.2°C in the plastic restrainer.

The thermic response to morphine was dependent on the dose of morphine and type of restraint. Figure 1 shows the mean time-response curves for the saline, hyperthermic and hypothermic morphine doses. Saline causes the least decline in rectal temperature in the wire-mesh restrainer, more in the rigid plastic restrainer and the greatest decrease in the confined rats (Fig. 1A). The actual rectal temperatures 4 hr after a saline injection were not different whether the rats were in the wire-mesh restrainer (37.1±0.2°C), plastic restrainer (37.4±0.2°C) or confined to the mouse pan (37.4±0.2°C).

The lowest dose of morphine (3.8 mg/kg) caused hyperthermia in all groups (Fig. 1B, Table 1). The increase in temperature in the rats which were confined in the mouse pan and restrained in rigid plastic were very similar in magnitude and time course, with the maximum temperature reached at 1.5 hr. Rats restrained in the wire-mesh lost approximately 0.5°C before the rise to maximum hyperthermia at approximately 2 hr. When a higher dose of morphine (7.5 mg/kg) was given the rats in the wire-mesh restrainer became 1°C hypothermic at 1 hr and then regained body temperature with a slight overshoot after 2 hr (Tables 1 and 2). The rats in

the plastic restrainer maintained body temperature initially and slowly increased temperature to approximately 1°C above baseline at 3 hr. The rats which were confined became hyperthermic almost immediately and then rectal temperatures slowly decreased to 1°C below baseline at 4 hr after injection. At 15 mg/kg of morphine similar responses occurred except that rats in the wire-mesh restrainer became more hypothermic than at 7.5 mg/kg. Maximum decreases in rectal temperature caused by a larger morphine dose (30 mg/kg) was approximately 6°C in rats restrained in the wire-mesh restrainers, 4°C in the rats restrained in the plastic restrainers and 1.5°C in the rats which were confined to the mouse pan (Fig. 1C). The response to the highest dose (45 mg/kg) was similar. When compared to saline control, morphine caused hyperthermia (Table 1) in the confined rats at all doses except 45 mg/kg. Morphine caused hyperthermia in the rats restrained in the plastic at doses between 3.8 and 15 mg/kg and only at 3.8 and 7.5 mg/kg in the rats restrained in the wire-mesh. Morphine administration resulted in hypothermia when compared to saline (Table 2) at 30 and 45 mg/kg only in both groups of restrained rats.

The differences in hypothermic response due to the type of restraint are most apparent in the dose-response curves (Fig. 2). The maximum hypothermic response in each method of restraint occurred at approximately the same morphine dose, however, the threshold dose for a hypothermic response is slightly less in the wire mesh restraint.

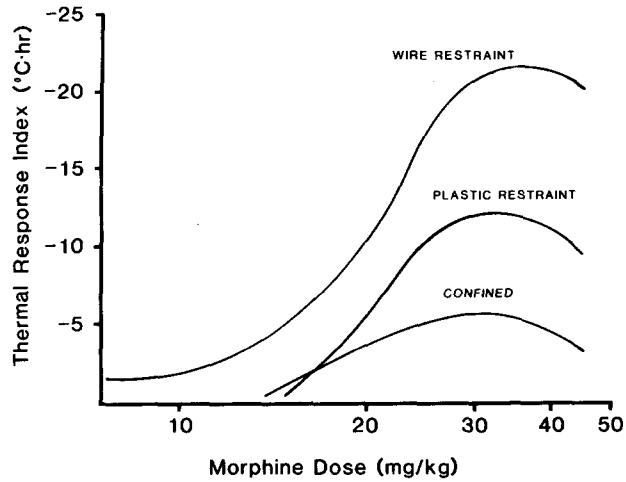


FIG. 2. Hypothermic response indices to morphine during different types of restraint. Graphic expression of same data as Table 2.

## DISCUSSION

This study shows that the effects of restraint on the thermoregulatory response to morphine are complicated and that factors other than stress are involved in the thermic response to morphine in restrained rats. These results are consistent with studies which showed that the same dose of morphine can produce hypothermia in restrained rats and hyperthermia in unrestrained rats [16,23] and suggest that the interference by restraint with behavioral or postural effects of morphine are partially responsible for this effect.

In this study we interfered with the expression of a morphine-induced hunched posture by restraining some of the rats. Body temperature is a balance between heat production and heat loss [3,9] and mammals can fairly rapidly control their temperatures by adjustments in behavior and autonomic function [7]. Among these adjustments are postural changes, piloerection, panting, saliva spreading, changes in vascular perfusion and shivering [2,7]. It cannot be determined whether the hunched posture is a direct behavioral effect of morphine or simply a response elicited to prevent the hypothermia induced by morphine.

In agreement with previous studies [15, 16, 23], morphine produced only hyperthermia in rats which were not restrained. Morphine produced both hyperthermia and hypothermia in restrained rats depending on dose. Restraint in the wire-mesh, which did not allow heat accumulation, caused an even greater fall in body temperature than occurs in the rats restrained in plastic.

Restraint may inhibit postural adjustments required to retain heat. The abnormal elongated posture forced by cylindrical restraint cages may increase heat loss by exposing the thinly furred ventral surface of the rat, because restraint produced greater hypothermia in a cold environment when compared to unrestrained rats and heat loss was decreased when rats were restrained in a more normal huddled posture [1,2]. The degree of restraint was equivalent between wire mesh and plastic restrainers, therefore, differences in morphine response in the two conditions were due to differences in heat conduction rather than to restraint stress as previously suggested [8,22]. Previous studies have shown that hypothermia produced by morphine is inversely related to the ambient temperature [18].

The stress of restraint alone cannot explain differences between restrained and non-restrained rat responses. It has been shown that placing a rat in a novel environment, similar to our mouse pan, caused an increase in plasma corticosteroids and a decrease in hypothalamic norepinephrine [11]. At 2 hours after the start of this stress the changes induced by a novel environment and restraint in a plastic restrainer were identical. In our study, rats were placed in the conditions for temperature measurement 2–3 hours before injection of morphine or saline in an attempt to adapt them to their environments. Therefore, differences in stress were probably not large so the thermic response differences to morphine are not easily explained by stress alone.

If stress affected the thermoregulatory response to morphine by releasing endogenous opioids as suggested [8,22], one would expect the morphine to have a different potency when combined with restraint. Although a valid $ED_{50}$ cannot be calculated, our study shows that the dose of morphine that produces an approximate half maximal response is the same regardless of restraint, but that the maximal response is dependent on the restraint condition. Therefore, it does not seem likely that a stress-induced release of endogenous opioids is responsible for the differences in morphine response.

In summary, we have found that the thermoregulatory response to morphine differs both quantitatively and qualitatively depending on the condition of restraint. In rats which were confined to a mouse pan, morphine caused mainly hyperthermia. In rats which were restrained, morphine caused hyperthermia at low doses and hypothermia at high doses. With an identical degree of restraint, the hypothermic responses to morphine were dependent on the heat loss capability of the rats in the restrainer, since hypothermic responses to morphine were increased in the wire-mesh restrainer. These results suggest that the effect of

restraint on thermic responses to morphine is due to a specific postural action of morphine. These results are consistent with the idea that the effects of restraint on morphine thermoregulatory responses are due to interference with the postural thermoregulation utilized by non-restrained rats.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the excellent assistance of A. M. Peterson. Supported by USPHS grants AG01289 and NS15420.

## REFERENCES

1. Bartlett, R. G., Jr. and F. H. Quimby. Heat balance in restraint (emotionally) induced hypothermia. *Am J Physiol* **193:** 557–559, 1958.
2. Bartlett, R. G. Jr., N. Mantel, G. L. Foster and P. Bernstein. Core to surface thermal gradients in the rat at several environmental temperatures. *Am J Physiol* **193:** 541–546, 1958.
3. Bligh, J. The central neurology of mammalian thermoregulation. *Neuroscience* **4:** 1213–1236, 1979.
4. Burks, T. F. and G. C. Rosenfeld. Narcotic Analgesics. In: *Body Temperature*, edited by P. Lomax and E. Schonbaum. New York: Marcel Dekker, 1979, p. 531.
5. Clark, W. G. Influence of opioids on central thermoregulatory mechanisms. *Pharmacol Biochem Behav* **10:** 609–613, 1979.
6. Clark, W. G. and H. R. Cumby. Hyperthermic responses to central and peripheral injections of morphine sulphate in the cat. *Br J Pharmacol* **63:** 65–71, 1978.
7. Crawshaw, L. I. Temperature regulation in vertebrates. *Annu Rev Physiol* **42:** 473–491, 1980.
8. French, E. D. Dexamethasone blocks morphine-induced hypothermia in restrained rats. *Life Sci* **25:** 1583–1590, 1979.
9. Hammel, H. T. Regulation of internal body temperature. *Annu Rev Physiol* **30:** 641–710, 1968.
10. Hermann, J. B. The pyretic action on rats of small doses of morphine. *J Pharmacol Exp Ther* **76:** 309–315, 1942.
11. Keim, K. L. and E. B. Sigg. Physiological and biochemical concomitants of restraint stress in rats. *Pharmacol Biochem Behav* **4:** 289–297, 1976.
12. Lomax, P. Drugs and body temperature. *Int Rev Neurobiol* **12:** 1–43, 1970.
13. Lotti, V. J., P. Lomax and R. George. N-allylnormorphine antagonism of the hypothermic effect of morphine in the rat following intracerebral and systemic administration. *J Pharmacol Exp Ther* **150:** 420–425, 1965.
14. Martin, G. E. and J. E. Morrison. Hyperthermia evoked by the intracerebral injection of morphine sulphate in the rat: the effect of restraint. *Brain Res* **145:** 127–140, 1978.
15. Martin, G. E. and N. L. Papp. Effect on core temperature of restraint after peripherally and centrally injected morphine in Sprague-Dawley rat. *Pharmacol Biochem Behav* **10:** 313–315, 1979.
16. Martin, G. E., A. T. Przbylik 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.
17. McDougal, J. N., P. R. Marques and T. F. Burks. Reduced tolerance to morphine thermoregulatory effects in senescent rats. *Life Sci* **28:** 137–145, 1982.
18. Paolino, R. M. and B. K. Bernard. Environmental temperature effects on the thermoregulatory response to systemic and hypothalamic administration of morphine. *Life Sci* **7:** 857–863, 1968.
19. Reynolds, A. K. and L. O. Randall. *Morphine and Allied Drugs*. London: Oxford University Press, 1957, p. 89.
20. Rosenfeld, G. C. and T. F. Burks. Single-dose tolerance to morphine hypothermia in the rat: differentiation of acute from long-term tolerance. *J Pharmacol Exp Ther* **202:** 654–659, 1977.
21. Rudy, T. A. and T. L. Yaksh. Hyperthermic effects of morphine: set point manipulation by direct spinal action. *Br J Pharmacol* **61:** 91–96, 1977.
22. Stewart, J. and R. Eikelboom. Interaction between the effects of stress and morphine on body temperature in rats. *Life Sci* **28:** 1041–1045, 1981.
23. Trzcinka, G. P., J. M. Lipton, M. Hawkins and W. G. Clark. Effects on temperature of morphine injected into the preoptic/anterior hypothalamus, medulla oblongata, and peripherally in unrestrained and restrained rats. *Proc Soc Exp Biol Med* **156:** 523–526, 1977.
24. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill, 1962, p. 201.