Pharmacology Biochemistry & Behavior, Vol. 16, pp. 23–27, 1982. Printed in the U.S.A.

Effects of Intracerebroventricular Capsaicin on Thermoregulatory Behavior in the Rat

B. DIB

Universite Claude Bernard, Faculte de Medecine Lyon-Sud
Laboratoire de Physiologie, L.A. C.N.R.S. N° 181, B.P. 12-69600 Oullins France

Received 13 June 1981

DIB, B. *Effects of intracerebroventricular capsaicin on thermoregulatory behavior in the rat.* PHARMAC. BIOCHEM. BEHAV. **16**(1) 23–27, 1982.—To clarify the action of capsaicin on the thermoregulatory system of rat, behavioral and autonomic responses were studied following intracerebroventricular (ICV) injection. Rats were chronically implanted with a lateral cerebral ventricular guide cannula. After the recovery period they were placed in a climatic chamber at ambient temperature (T_a) of 20, 30 or 35°C. In the first series of experiments, they had access to a lever which activated a fan that drew cool outside air into the chamber. After ICV capsaicin (23 μg), the rats increased bar-pressing behavior for fresh air at T_a ranging from 20°C to 35°C. In the second series of experiment, the rats had no access to fanning. ICV capsaicin produced a fall in rectal and hypothalamic temperature (T_{hy}) and an increased in cutaneous temperature. These changes depended on T_a. At a T_a of 30°C T_{hy} fell slightly (mean of 0.2±0.16°C). At a T_a of 20°C T_{hy} fell to a mean of 1°C±0.17°C. The conclusion drawn is ICV capsaicin activated behavioral as well as autonomic thermoregulatory heat-loss responses. The effect of capsaicin resembles the effect of local heating of the hypothalamus. However, since hypothalamic temperature decreased the drug may have lowered the thermal set point, or excited directly hypothalamic warm-sensitive neurons.

Capsaicin Intracerebroventricular Thermoregulatory Behavior Body temperature

CAPSAICIN injected intraperitoneally or subcutaneously into mice, rats or guinea-pigs produces an immediate fall in body temperature [15, 16, 18, 19, 22]. The fall in rectal temperature is brought about by autonomic heat loss responses such as vasodilatation, salivation and reduction in metabolic rate [23]. Thermoregulatory heat-loss responses can also be induced experimentally by a temperature rise in the hypothalamus [1, 2, 4, 6]. The aim of the present experiment was therefore two-fold: (1) to explore the effect of capsaicin on behavioral heat loss responses; (2) to investigate whether capsaicin could act indirectly on thermoregulatory heat loss responses by a rise in the hypothalamic temperature. To answer these questions, hypothalamic temperature and behavioral thermoregulatory responses were measured following an intracerebroventricular injection of capsaicin.

METHOD

Chronic guide cannulae of stainless steel tubing, 0.5 mm inner diameter, were implanted in a lateral ventricle of nineteen wistar albino rats, 320–360 g, under pentobarbital anaesthesia. Stereotaxic coordinates were: l=2 mm, V=3.5 mm, A=6 to 6.5 mm [3]. The tube was used for intracerebroventricular (ICV) injection of capsaicin and saline. Polyethylene guide tubes of 0.7 mm external diameter were implanted to the preoptic area, 1 mm from the midline in the left side, to measure hypothalamic temperature. After a recovery period of at least one week, correct placement of the ventricular cannulae was confirmed one day before the experiment by an infusion of 1 μl of a solution containing 1 ng of human angiotensin II (Beckman). Given intraventricularly this octapeptide induces an immadiate drinking response [10]. Thirteen rats with the cannulae correctly placed were used in the experiment. Five rats showing no drinking response to angiotensin were excluded from the study. Histologic examination of the brain confirmed that in these cases the cannulae were placed outside the lateral ventricle. The rats were placed in a controlled ambient temperature (T_a) and injected with isotonic saline (NaCl) (45 μg in 5 μl) and/or capsaicin (23 μg in 5 μl) in the lateral ventricle. Capsaicin was dissolved by means of ethanol and tween 80 in physiological saline, as described by Jancso *et al.* [17]. Preliminary experiments had shown that higher doses of capsaicin could kill the rats and that lower doses might have no effect on thermoregulation. No systematic study of the dose effect has previously been attempted. Controlled T_a was obtained by a double wall copper cylinder. The space between the copper walls was filled with constantly stirred water at regulated temperatures. Hot, cool and cold wall temperatures provided air temperature around the rat of respectively 35, 30 or 20°C. In order to measure thermoregulatory behavior against heat, the cage was equipped with a lever 5.5 cm long and 0.5 cm broad. A bar press by the rat switched on a fan placed against a window, which the draught opened. The fresh air reward was therefore outside air at room temperature (18–20°C). The wind at 5 mps^{-1} was delivered as long as the

animal pressed the lever. Bar-pressing time in seconds (sec) was recorded electronically on a digital counter. In order to learn how to use the lever, the rats were placed in the climatic chamber for 1 hour each day at the hot ambient temperature (35°C) two or three days before the experiments.

RECORDING OF TEMPERATURES.

Three body temperatures were recorded using thermocouples; one in the hypothalamus (T_{hy}), through the polyethylene guide tubes; one in the colon, 6 cm from the anus (T_{re}); and the last on the dorsal side at the base of the tail (T_s). This last represents the cutaneous temperature. These thermocouples were wound in a spiral, thus enabling the rats to move freely in the climatic chamber [9].

EXPERIMENT 1

The behavioral thermoregulatory responses of seven rats were measured, together with T_{re} and T_s for 100 minutes at T_a of 20, 30, and 35°C. Immediately on entering the climatic chamber the rats had access to fanning. After 20 min the rats received 5 μl of NaCl. Bar-pressing was measured after this injection. Fifty minutes after the NaCl injection they received 23 μg of capsaicin in 5 μl, and bar-pressing measurement was continued over these 50 minutes.

EXPERIMENT 2

In six rats intracerebral injections were identical. The rats were maintained at T_a of 20 and 30°C and had no access to fanning. This experiment allowed of recording T_{hy}. T_{re}, and T_s continuously with no perturbation by thermoregulatory behavior.

During the first 15 or 20 minutes the rats adapted themselves to the climatic chamber. They then received 5 μl of NaCl. Twenty or thirty minutes after NaCl they received 23 μg of capsaicin in 5 μl and temperature measurement was continued for 30 to 40 mn.

RESULTS

Body temperatures were measured every five minutes. In Figs. 1, 2 and 3, the exploration period (15 min), before NaCl injection of experiment is not shown.

EFFECTS OF INTRACEREBROVENTRICULAR CAPSAICIN ON BEHAVIORAL FANNING AND BODY TEMPERATURE

Cutaneous and Rectal Temperatures

Ambient temperature 20°C: Figure 1 presents mean results for four rats. After the capsaicin injection T_{re} declined as T_s increased. Mean decrease in T_{re} was 1.2±0.20°C, and mean increase in T_s 2.1±4°C. The injection of NaCl produced little change in T_{re} or T_s. The effect of capsaicin was statistically significant both on T_{re} ($p<0.01$), and T_s ($p<0.05$).

Ambient temperature 30°C: Figure 2 presents mean results for four rats. After the capsaicin injection, T_{re} decreased as T_s increased. Mean decrease in T_{re} was 0.5±0.3°C, and mean increase in T_s 1±0.3°C. The injection of NaCl had no effect on T_s or T_{re}. The effect of capsaicin was not statistically significant on either T_{re} $p>0.1$, and T_s $p>0.2$.

Ambient temperature 35°C: Figure 3 presents mean results for five rats. After the capsaicin injection, T_{re} and T_s decreased. Mean decrease in T_{re} was 0.5±0.3°C, and mean decrease in T_s 0.5±0.5°C. The injection of NaCl had no effect on T_s or T_{re}. The effect of capsaicin was not statistically significant $p>0.20$.

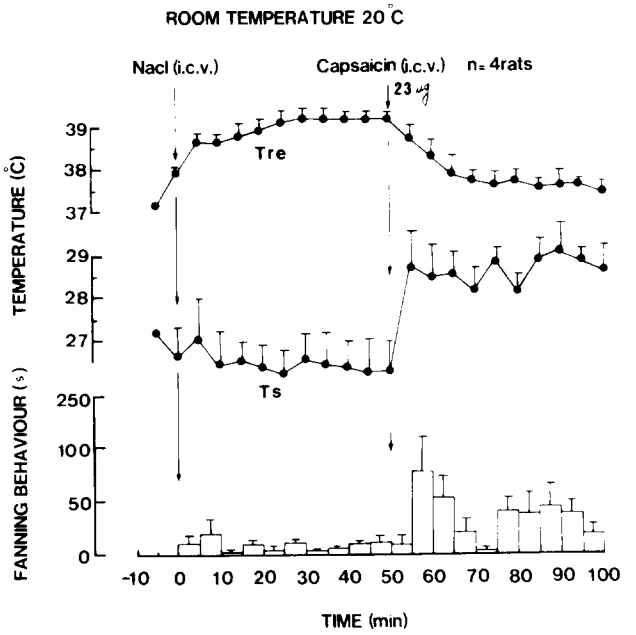

FIG. 1. T_a=20°C. Evolution of mean T_{re} and T_s following a single ICV injection of capsaicin followed by NaCl. Underneath, mean time (s) spent in bar-pressing after these injections. Vertical lines indicate S.E.M..

Fanning Behavior

Ambient temperature 20°C: Figure 1 presents mean time (sec) spent in bar-pressing to obtain a cool wind. This was, considerably higher in the 50 minutes following injection of capsaicin. After NaCl injection little bar-pressing occurred. Mean bar pressing time increased for 71.8±20 sec after NaCl, to 335±90.3 sec after capsaicin. This difference was statistically significant ($p<0.05$).

Ambient temperature 30°C: Figure 2 presents mean time spent in bar pressing increased from 434.8±25.8 sec over 50 minutes following NaCl injection, to 945±116.2 sec over 50 minutes after capsaicin. The difference was statistically significant ($p<0.01$).

Ambient temperature 35°C: Figure 3 presents mean time spent in bar pressing increased from 667.8±90 sec over 50 minutes following NaCl injection to 1139.5±164.7 sec over 50 minutes following capsaicin injection. The difference was statistically significant ($p<0.05$).

Fig. 4 compares the effect of capsaicin and NaCl on heat loss behavior at the ambient temperature of 20, 30, and 35°C. Some rats were used at more than one ambient temperature.

EXPERIMENT 2: EFFECT OF INTRACEREBROVENTRICULAR CAPSAICIN ON HYPOTHALAMIC TEMPERATURE

In this experiment, T_{hy} as well as T_{re} and T_s were recorded at T_a of 20 and 30°C in the absence of thermoregulatory behavior.

Ambient temperature 20°C: Figure 5 presents mean re-

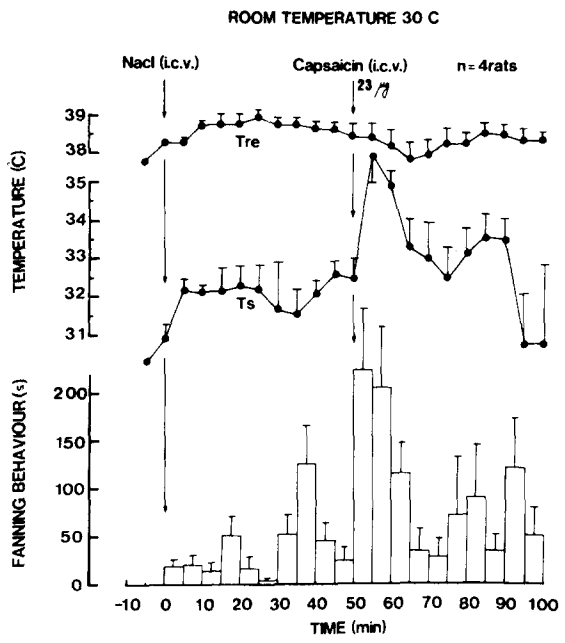

FIG. 2. T_a=30°C (legend identical to Fig. 1).

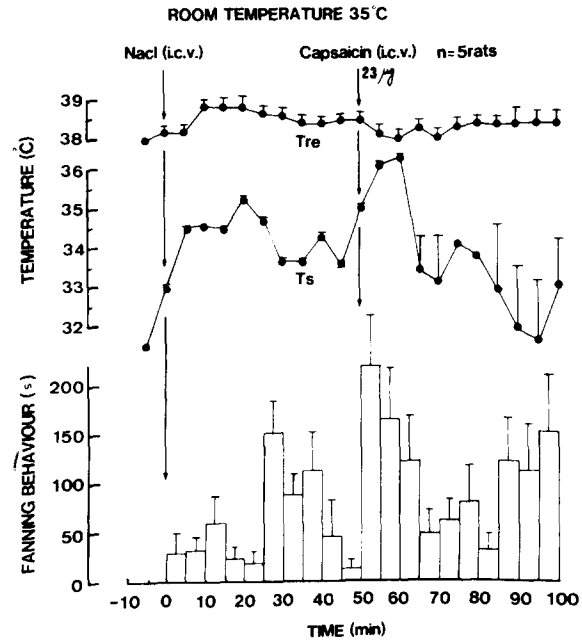

FIG. 3. T_a=35°C (legend identical to Fig. 1).

sults for two rats. After NaCl injection, mean T_{re} was 38.6±0.06°C, mean T_{hy} 37.2±0.06°C, mean T_s 27.05±0.17°C. After capsaicin mean T_{re} fell to 37.1±0.14°C, mean T_{hy} fell to 36.2±0.1°C and mean T_s rose to 28.3±0.4°C.

The changes in T_{re} and T_{hy} were statistically significant: $p<0.2$. However, the variation in T_s was not statistically significant: $p>0.2$.

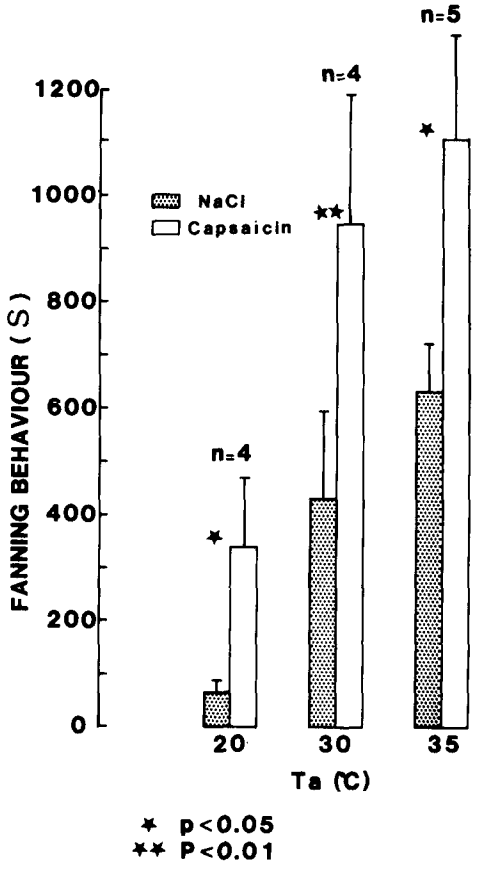

FIG. 4. Mean time (s) spent in bar-pressing at T_a of 20, 30, and 35°C. Each set of 2 columns represents a sequence of 100 mn experiment: 50 mn after NaCl (grey) 50 mn after capsaicin (white). "n" indicates the numbers of rats and experiments, asterisks indicate statistical level of significance (*$p<0.05$, **$p<0.01$, t-test).

Ambient temperature 30°C: Figure 6 presents mean results for four rats. After NaCl injection, mean T_{re} was 39±0.06°C, mean T_s 32.5±0.8°C, and mean T_{hy} 37.8±0.2°C. After capsaicin mean T_{hy} fell slightly to 37.6±0.2°C, mean T_{re} fell to 38.4±0.1°C and mean T_s rose to 34.9±0.34°C. The variations in T_{re} and T_s were statistically significant $p<0.01$. However, changes in T_{hy} were not statistically significant $p>0.2$.

In this study, after capsaicin injection a fall in T_{re} associated with a rise in T_s was observed. The rise in T_s was probably due to vasodilatation. This would increase cutaneous heat loss and may account for the decrease in T_{re}. This also confirms the activation of heat-loss mechanisms after capsaicin injection. A dose-dependant hypothermia associated with skin vasodilatation has also been reported [16, 18, 19, 22] after capsaicin was injected subcutaneously intraperitoneally or intrahypothalamically. At T_a of 20, 30 and 35°C, T_{re} and T_s changed rapidly after capsaicin injection. But when the rats pressed the lever to obtain a cool wind their body temperature returned to normal pre-injection values. After capsaicin injection significant differences in T_{re} and T_s were observed at 20°C only. This shows that at T_a of 20°C the rats

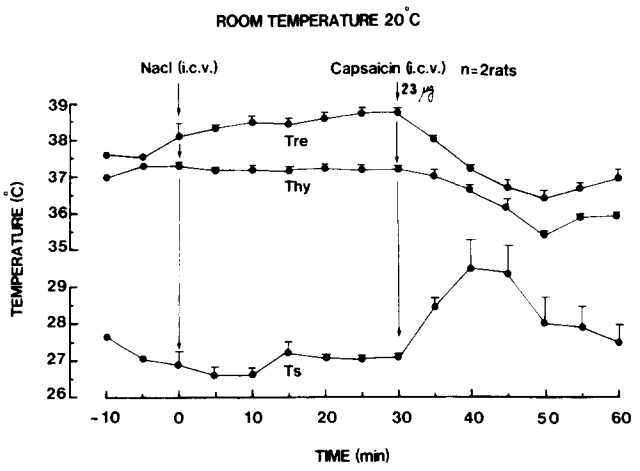

FIG. 5. T_a=20°C. Evolution of mean T_{re}, T_s and T_{hy} following a single ICV injection of capsaicin followed by NaCl. Vertical lines indicate S.E.M..

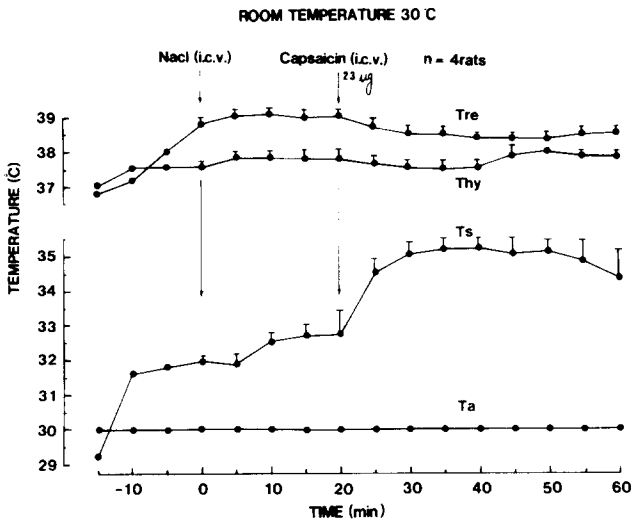

FIG. 6. T_a=30°C (legend identical to Fig. 5).

were regulating their body temperature essentially by autonomic thermoregulatory heat-loss responses.

At T_a of 30 or 35°C autonomic responses existed since T_s increased rapidly after capsaicin. However T_s returned to pre-injection level, due to the intense fanning activity. The efficacy of the heat-loss behavior explains why the mean changes in T_{re} and T_s are not statistically significant. It may be concluded that after capsaicin at this high T_a rats regulated their body temperature essentially by behavioral means. A similar pattern of heat-loss responses has been obtained by local heating of the hypothalamus [1, 2, 4, 5, 6, 7, 8, 11, 12]. The present work shows that capsaicin does not raise T_{hy}. On the contrary T_{hy} fell by 0.2 and 1.0°C at T_a of 30 and 20°C respectively. It may therefore be concluded that ICV capsaicin did not raise T_{hy} but rather excited central neurons that activated behavioral as well as autonomic heat-loss responses. This led to a fall in T_{re} and activation of fanning behavior. It must may be hypothesized that excitation of central neurons may result either from a falling thermal set point [5] or from a sensitization of warm sensitive neurons in the hypothalamus. This second hypothesis is suggested by the electrophysiological studies of Nakayama *et al.* [21] who observed that warm-sensitive neurons in the hypothalamus were sensitized in their response to local hypothalamic heat after a subcutaneous injection of capsaicin. However, in their experiment the action of capsaicin on hypothalamic neurons resulted perhaps indirectly from a modification of subcutaenous afferents to hypothalamus. The capsaicin sensitive-neurons in the central nervous system might correspond to the heat sensitive neurons found in the hypothalamus in different species by the other authors [13, 14, 20].

ACKNOWLEDGEMENTS

We wish to express our thanks to Professors M. Cabanac and R. Duclaux for reading and revising the manuscript.

This work was supported by the Centre National de la Recherche Scientifique (L.A.N.° 181) and the Institut National de la Santé et de la Recherche Médicale (I.N.S.E.R.M., A.T.P. 80-79-112 and Contrat Libre 79-1-316-6).

REFERENCES

1. Adair, E. R. Behavioral temperature regulation in squirrel monkey unilateral versus bilateral preoptic thermal stimulation. *Israel J. med. Sci.* **12:** 1033–1035, 1976.
2. Adair, R. E., J. U. Casby and J. A. J. Stolwijk. Behavioral temperature regulation in the squirrel monkey: changes induced by shifts in hypothalamic temperature. *J. comp. physiol. Psychol.* **72:** 17–27, 1970.
3. Albe-Fessard, D., F. Stutinsky and S. Libouban. *Atlas Stéréotaxique du Diencéphale du rat blanc.* Paris: Editions C.N.R.S., 1971.
4. Baldwin, B. A. and D. L. Ingram. The effect of heating and cooling the hypothalamus on behavioural thermoregulation in the pig. *J. Physiol., Lond.* **191:** 375–392, 1967.
5. Cabanac, M. Temperature regulation. *A. Rev. Physiol.* **37:** 415–439, 1975.
6. Cabanac, M. and B. Dib. Behavioural responses to hypothalamic heating in the rat. Submitted, *J. Physiol., Lond.* 1981.
7. Carlisle, H. J. Behavioural significance of hypothalamic temperature sensitive cells. *Nature* **209:** 1324–1325, 1966.
8. Carlisle, H. J. and M. J. Laudenslager. Observations on the thermoregulatory effects of preoptic warming in rats. *Physiol. Behav.* **23:** 723–732, 1979.
9. Dib, B. Continuous perfusion under pressure using several tubes in the freely behaving rat. *Physiol. Behav.* **24:** 177–178, 1980.
10. Epstein, A. M., J. T. Fitzsimons and B. J. Rolls. Drinking induced by injection of angiotensin into the brain of the rat. *J. Physiol., Lond.* **210:** 457–474, 1970.
11. Gale, C. C., M. Mathews and J. Young. Behavioral thermoregulatory responses to hypothalamic cooling and warming in baboons. *Physiol. Behav.* **5:** 1–6, 1970.
12. Hammel, H. T. Regulation of internal body temperature. *A. Rev. Physiol.* **30:** 641–710, 1968.

13. Hardy, J. D., R. F Hellon and K. Sutherland. Temperature-sensitive neurons in the dog's hypothalamus. *J. Physiol., Lond.* **175:** 242–253, 1964.
14. Hellon, R. F. Thermal stimulation of hypothalamic neurons in unanaesthetized rabbits. *J. Physiol., Lond.* **193:** 381–395, 1967.
15. Jancsó-Gábor, A. Some data to the pharmacology of histamine releasers. (Adatok a hisztamin-felszabadito anyagok pharmakologiajahoz). Dissertation, Szeged, 1947.
16. Jancsó, N. *Speicherung Stoffanreicherung in Retikuloendothel und in der Niere.* Budapest: Akademiá Riadó, 1955.
17. Jancsó, N., A. Jancsó-Gabor and J. Szolcsanyi. Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. *Br. J. Pharmac. Chemother.* **31:** 138–151, 1967.
18. Jancsó-Gábor, A., J. Szolcsányi and N. Jancsó. Irreversible impairment of thermoregulation induced by capsaicin and similar pungent substances in rats and guinea-pigs. *J. Physiol., Lond.* **206:** 495–507, 1970.
19. Jancsó-Gabor, A., J. Szolcsányi and B. Jancsó. Stimulation and desensitization of the hypothalamic heat sensitive structures by capsaicin in rats. *J. Physiol., Lond.* **208:** 449–459, 1970.
20. Nakayama, T., H. T. Hammel, J. D. Hardy and J. S. Eisenman. Thermal stimulation of electrical activity of single units of the preoptic region. *A. Rev. Physiol.* **204:** 1122–1126, 1963.
21. Nakayama, T., M. Suzuki, Y. Ishikawa and A. Nishio. Effects of capsaicin on hypothalamic thermo-sensitive neurons in the rat. *Neurosci. Lett.* **7:** 151–155, 1978.
22. Rabe, L. S., S. H. Buck, L. Moreno, T. F. Burks and N. Dafny. Neurophysiological and thermoregulatory effects of capsaicin. *Brain. Res. Bull.* **5:** 755–758, 1980.
23. Szolcanyi, J. and A. Jancso-Gabor. Capsaicin and other pungent agents as pharmacological tools in studies on thermoregulation. In: *The Pharmacology of Thermoregulation*, edited by E. Schonbaum and P. Lomax. Basel: Karger, 1972, pp. 395–409.