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Effects of Intrathecal Capsaicin on Autonomic and Behavioral Heat Loss Responses in the Rat

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DIB, B. *Effects of intrathecal capsaicin on autonomic and behavioral heat loss responses in the rat.* PHARMACOL BIOCHEM BEHAV **28**(1) 65–70, 1987.—Capsaicin and Tween 80 were injected into the lumbar subarachnoid space of rats via a chronic cannula, and the thermoregulatory effects compared. The rats were placed in a climatic chamber at an ambient temperature (T_a) of 20 and 30°C. In the first series of experiments the rats had no access to the fan lever. Intrathecal (IT) capsaicin injection produced a fall in rectal temperature, with a rise in cutaneous temperatures due to vasodilation. On the contrary, IT or the intraperitoneal (IP) Tween 80 injection route had no effect on body temperature. In addition capsaicin-administered IP induced a fall in spinal cord temperature (T_{sp}). In the second series of experiments the rats had access to a lever activating a fan that drew cool outside air into the climatic chamber. After IT capsaicin injection, the rats increased bar-pressing behavior for fresh air. This was significant at both T_a 20 and 30°C. The results tend to support the hypothesis of capsaicin action somewhere on the thermal afferent pathways. Furthermore, it is possible that the action of capsaicin on thermoregulatory behavior is mediated by the release of substance P from primary afferent terminals.

Intrathecal Capsaicin Behavior Hypothermia

IN rats intracerebroventricular (ICV), subcutaneous (SC) or intravenous (IV) injection of capsaicin produced a fall in body temperature [7, 12, 13, 15, 23]. This was brought about by autonomic heat loss responses such as vasodilatation, salivation and the reduction of mobilization in metabolic rate [4, 8, 17, 22]. Behavioral heat defenses, such as body extension, increased escape reaction from a warm chamber [22] and increased skin cooling operant behavior [7, 14, 15] were also enhanced.

Thermoregulatory heat-loss responses can also be induced by a temperature rise in the hypothalamus [1, 3, 5] and in the spinal cord [10,21]. It is known that the spinal cord, among other central nervous system (CNS) locations, contains a population of thermodetectors [21], and that these may respond not only to temperature but also to drugs [18]. In our previous study [7] we show that rats increased bar-pressing for cool air immediately after acute ICV injection of capsaicin. We wished to find out whether, under the same conditions, the rats would increase bar-pressing after intrathecal (IT) injection of capsaicin.

METHOD

Subjects and Surgery

Semi-sterile instruments were used. Polyethylene tubing, 0.7 mm in external diameter and 0.3 mm in internal diameter, was implanted in the IT space of 42 OFA albino rats, weighing 300–330 g and under nembutal anesthesia (35 mg/kg). The intravertebral disc between C8 and T1 and then the dura were removed, using forceps, without harming the spinal cord. The 7.5 cm-long cannula was inserted from T1 down to L3 and fixed to the processus transversus T1 according to the method of Dib [9]. The rats were allowed a recovery period of at least one or two weeks, and were used in experiments only when their appearance and behavior seemed perfectly normal. Six rats were discarded 3–4 days after surgery, due to motor impairment in the hind legs. The rats were petted and handled every day to reduce the emotional effects of the imminent experiments to a minimum.

To reduce the influence of the nychthemeral body temperature cycle each experiment was conducted at the same time of the day for each rat.

Some rats were used in more than one experiment. These rats were first used for Tween 80 injection and then used for capsaicin injection.

Climatic Chamber

For test sessions the rats were placed in a double-walled copper cylinder. The temperature in the cylinder was maintained at 20 and 30°C by water at the appropriate temperature circulating between the walls of the cylinder [6].

Each experimental session was divided into 3 parts: 30 min, acclimation period; 30 min, control; 30 min, experiments after drug injection. The rat received a sterile solution of 40 μg of capsaicin in 10 μl or 10% of Tween 80 in 10 μl. All IT injections were made in the climatic chamber without removing the rats.

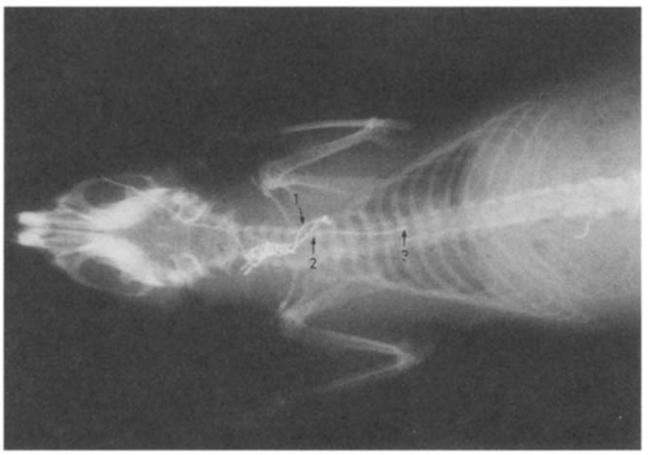

FIG. 1. Radiograph of a rat showing the thermocouple inserted before the experiment in the cannula implanted intrathecally. Black arrow 1 indicates the steel wire used to fix the cannula after implantation at the processus T1. Black arrows 2–3 indicate the zone of thermocouple insertion, from T1 to T5.

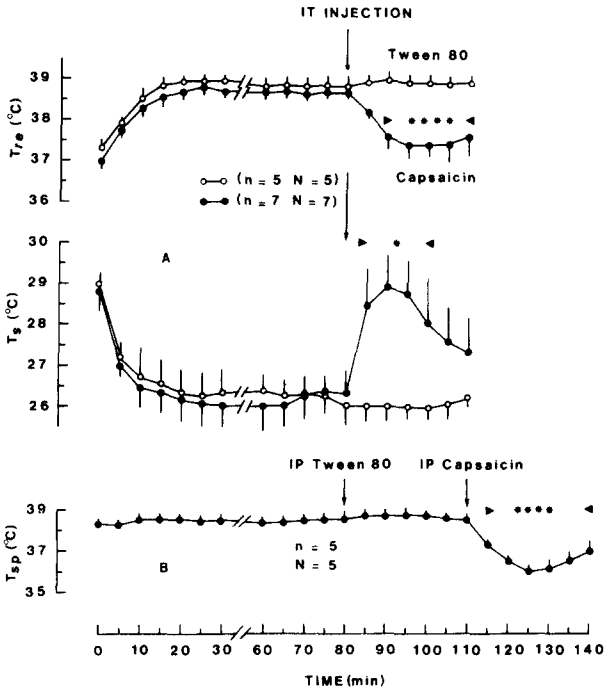

FIG. 2. T_a=20°C. Rats having no access to bar-pressing. Evolution of mean (±SE) T_{re} and T_s following a vehicle or drug injection. Figure 2A: shows the evolution of mean (±SE) T_{re} and T_s following a single intrathecal (IT) injection of capsaicin and Tween 80: n indicates the numbers of rats and N indicates the numbers of experiments (one experiment per rat). Asterisks indicate level of significant difference between post-injection mean temperature and the mean temperature at the temperature at the time of injection. Figure 2B: shows the evolution of mean (±SE) of spinal temperature (T_{sp}) following intraperitoneal (IP) injection of Tween 80 and capsaicin injection. IP capsaicin injection produced a significant difference in T_{sp}. Student's impaired t-test; *$p<0.05$; **$p<0.02$, ***$p<0.01$, ****$p<0.001$.

Capsaicin was dissolved in vehicle (10% Tween 80, 10% ethanol in physiological saline solution v/v, as described by Jancso, Jancso-Gabor and Szolcszanyi [15].

Recording of Temperatures

Two body temperatures were recorded from thermocouples placed in the rat: one in the colon 6 cm from the anus (T_{re}), and one on the dorsal side 1 cm from the base of the tail (T_s).

Spinal cord temperature (T_{sp}) was also recorded. To avoid emotional stress the thermocouple was inserted in the IT chronic cannula 30 min before the experiment. The inserted thermocouple reached T_4–T_5 as shown in the radiographs (Fig. 1). The thermocouple remained in place during and after drug injections.

Thermocouples were wound in a spiral, thus enabling the rat to move freely in the climatic chamber [6].

Body temperatures and thermoregulatory behavior were recorded continuously, but results are given as a mean of all experiments for every 5 min.

Two experiments were conducted as follow:

Experiment 1: Effect of IT or IP Capsaicin or Tween 80 on Body Temperature With No Lever

This experiment was designed to test the central mechanism inducing the body temperature change by IT capsaicin or its vehicle.

The changes in T_{re} and T_s following IT injection of Tween 80 or capsaicin were studied for 110 min at T_a of 20°C in 12 rats. After a period of 80 min of equilibration of body temperature, these rats received a control IT injection of 10 μl of vehicle (Tween 80) or capsaicin. In 5 other rats at T_a 20°C T_{sp} was recorded for 140 min. After a period of 80 min of equilibration, these rats received IP 800 μl of Tween 80. Thirty min after this injection they received IP 400 μg of capsaicin in 800 μl.

Experiment II: Effect of Capsaicin or Tween 80 on Behavioral Thermoregulatory Responses, and Body Temperature

This experiment was designed to test the central mechanism inducing thermoregulatory behavior by IT capsaicin or its vehicle. Forty-four rats were used: (a) Fifteen for Tween 80 IT administration. (b) Eighteen for IT capsaicin injection (one rat per experiment). In this experiment some rats used for IT Tween 80 injection were again used for capsaicin IT injection.

To measure heat-loss behavior the climatic chamber was equipped with a lever 5.5 cm long and 0.5 cm broad. By pressing on a bar the rat switched on a fan placed against a window, with the air-vent open. Cool air at 18–20°C circulated through the climatic chamber at 5 m/s as long as the animal pressed the lever. Bar-pressing time in seconds (sec) was recorded electronically on a digital counter. Three to five days before the experiments the rats were placed in the climatic chamber for 1–2 hr each day at T_a 30 or 35°C. After

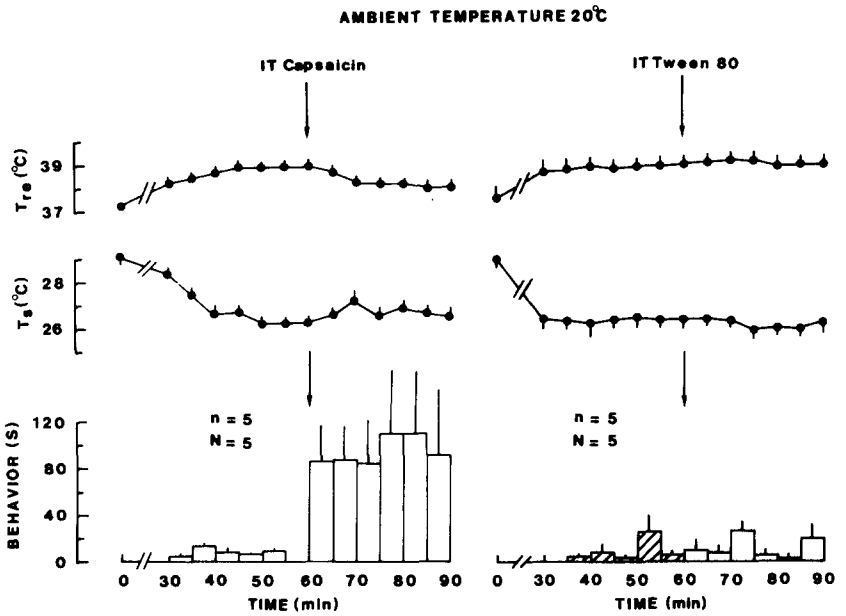

FIG. 3. T_a=20°C. Shows the evolution of mean (±SE) T_{re} and T_s following a single IT injection of capsaicin and Tween 80. Figure 3 left shows also the mean time (S) spent in bar-pressing after these injections. After IT capsaicin injection T_{re} fell as T_s rose significantly. The IT injection of Tween 80 did not produce any change in body temperature. (See caption to Fig. 2 for other meanings.)

this training period, and throughout the experiments, behavior T_{re} and T_s were recorded continuously for 90 min at T_a of 20 and 30°C. The rat had access to the lever immediately on entering the climatic chamber. The time spent in bar-pressing during the exploration period, from 0 to the 30th min of the experiment, is not shown in figures.

RESULTS

Experiment I: Effect of Capsaicin or Tween 80 on Body Temperature

In this experiment, T_{re}, T_s and T_{sp} were recorded at a T_a of 20°C, with no lever. Figure 2 shows the evolution of the mean body temperature before and after drug injection. Body temperature stabilized after 30–40th min. Figure 2A: before IT Tween 80 injection, the means of T_{re} and T_s were after 80 min respectively 38.7±0.04, and 25.9±0.33°C. For 30 min following IT Tween 80 injection, T_{re} rose by 0.3°C, while T_s fell by 0.2°C. It may therefore be concluded that the Tween 80 in which capsaicin was dissolved did not produce any change in body temperature.

Figure 2A, before IT capsaicin administration, the means of T_{re} and T_s after 80 min equilibration, were respectively, 38.6±0.1 and 26.3±0.6°C. After IT capsaicin injection T_{re} fell as T_s rose. As compared with the pre-injection temperature mean T_{re} fell and mean T_s rose for 30 min. The fall in T_{re} was statistically significant from the 10th min after injection till the end of the experiment (Student's impaired t-test, $p<0.001$). The T_{re} reached a minimum 1.2°C lower than the pre-injection temperature. The increase in T_s was statistically significant from the 5th min after injection to the 20th min of the experiment (Student's impaired t-test, $p<0.05$). The T_s reached a maximum 2.6°C higher than the pre-injection temperature. Spinal temperature (T_{sp}) was measured in 5 rats (Fig. 2B). In these rats T_{sp} stabilized after 20–30 min. Mean T_{sp} after 80 min was 38.4±0.13°C. Following the injection of 800 μl of Tween 80, T_{sp} rose slightly. T_{sp} rose 0.3°C higher than the pre-injection temperature. This rise was not statistically reliable. On the contrary, following IP of 400 μg of capsaicin T_{sp} fell by 3.9°C below the preinjection level. The fall in T_{sp} was statistically significant immediately after IP capsaicin injection till the end of the experiment (Student's impaired t-test, $p<0.01$).

Experiment II: Effects of IT Capsaicin or Tween 80 on Behavioral Thermoregulatory Responses and Body Temperature

Figures 3 and 4 shows mean T_{re} and T_s, and the time spent in bar-pressing to obtain a cool wind every 5 min at T_a of 20 and 30°C.

(1) Tail skin and rectal temperature. Ambient temperature 20°C. Figure 3 (left) presents mean results for 5 out of 9 rats. The means of T_{re} and T_s after 80 min were, respectively, 38.8±0.08°C and 26.3±0.11°C. Following injection of 40 μg of capsaicin mean T_{re} fell and mean T_s rose during 30 min, as compared with the pre-injection temperatures. The fall in T_{re} was statistically significant from the 5th min after injection till the end of the experiment (Student's impaired t-test $p<0.001$). T_{re} reached a minimum 1.2±0.15°C lower than the preinjection temperature. The rise in T_s was statistically significant from the 5th to the 10th min after injection (Student's impaired t-test, $p<0.01$). T_s reached a maximum 0.6°C higher than the pre-injection temperature. The small increase of T_s was perhaps due to the cool wind.

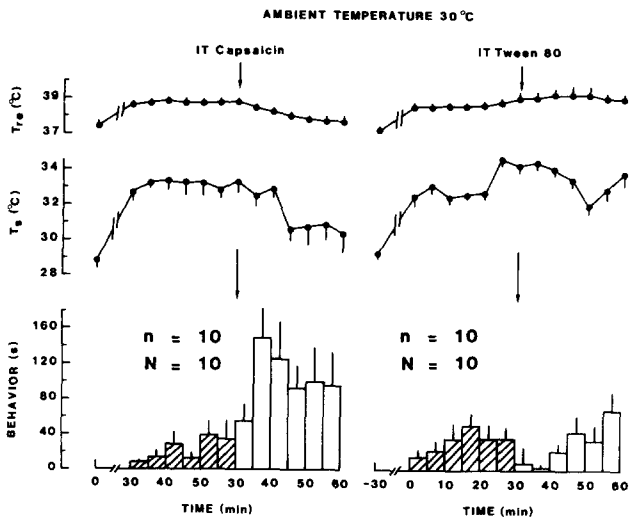

FIG. 4. T_a=30°C. After IT injection of capsaicin, T_{re} and T_s fell during 30 min. The fall in T_{re} is statistically significant from the 5th min after injection till the end of the experiment. The fall in T_s was statistically significant from the 5th min after injection till the end of the experiment. The IT injection of Tween 80 did not produce any significant change from the pre-injection temperature. (See caption to Fig. 2 for other meanings).

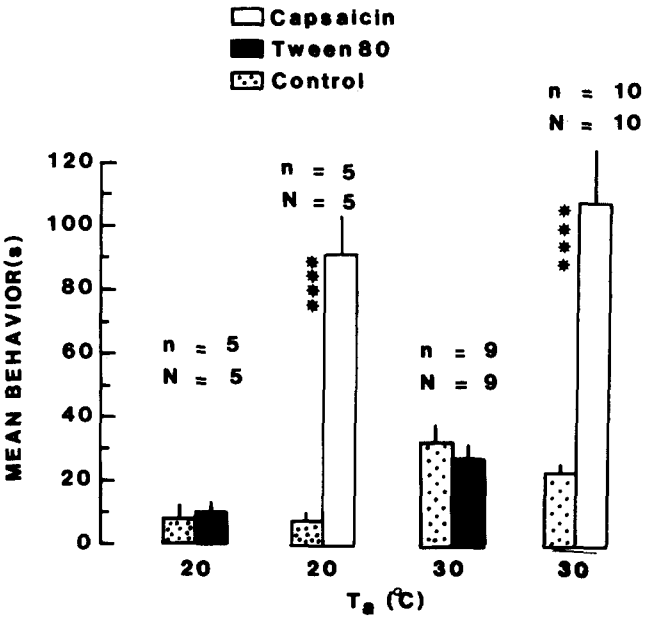

FIG. 5. Shows the general mean time (sec) spent in bar-pressing at T_a of 20 and 30°C before and after capsaicin or Tween 80 injection. Each set of 2 columns represents a sequence of 30 min experiment. 30 min during control period from minute 30 to 60, 30 min after capsaicin or Tween 80 injection (from minute 60 to 90). Asterisks indicate statistical level of significance: $p<0.002$. (See caption to Fig. 2 for other meanings.)

Figure 3 (right) shows that in 5 control rats the body temperature stabilized after the 20th min. The means of T_{re} and T_s after 60 min were respectively 38.8±0.3°C and 26.4±0.3°C. After Tween 80 injection T_{re} rose by 0.1, 0.2°C, while T_s fell by 0.4°C. When compared with the pre-injection temperature the body temperature change is not statistically significant.

Ambient temperature 30°C. Figure 4 (left) presents mean results for 10 rats. Body temperature stabilized after the 25th min. The means of T_{re} and T_s after 60 min were, respectively, 38.7±0.12°C and 33.3±0.6°C. Following injection of 40 μg of capsaicin, mean T_{re} and T_s fell during 30 min, as compared with the pre-injection temperatures. The fall in T_{re} was statistically significant from the 10th min after injection till the end of the experiment (Student's impaired t-test $p<0.001$). T_{re} reached a minimum 0.7°C lower than the pre-injection temperature. The fall in T_s was statistically significant from the 35th min after injection till the end of the experiment (Student's impaired t-test, $p<0.05$). The fall in T_s after capsaicin injection may be due to the cool wind. Figure 4 (right) shows that in 10 control rats body temperature stabilized after 20–30 min. The means of T_{re} and T_s after 60 min were respectively 39.2±0.14°C and 34.2±0.4°C. After Tween 80 injection T_{re} rose by 0.1°C, while T_s changed little. The small change in T_{re} and T_s is not statistically significant from the pre-injection temperature.

We have observed in the first and second experiments that T_{re} rose from 37.2±0.3°C at zero time to 39.06±0.18°C at the 30th min. The rise in T_{re} may be due to stress [2].

(2) Fanning behavior. Figure 3 presents mean time (sec) spent in bar-pressing to obtain a cool wind for a T_a of 20°C. Figure 3 (left): the injection of 40 μg of capsaicin stimulated this behavior in 5 rats out of 9. After capsaicin injection mean bar-pressing time rose from 6.0±1.5 sec to 94.0±9.5 sec. The mean bar-pressing time of the other 4 rats did not activate too much the fanning behavior and was not presented in the Fig. 3. After capsaicin injection mean bar-pressing time in these rats before and after capsaicin was respectively 9.07±2.01 sec and 7.15±3.0 sec. The effect of capsaicin is statistically significant (Mann and Whitney: U=1; $p<0.002$). Figure 3 (right): in control rats (n=5) the injection of 10% of Tween 80 did not stimulate this behavior too much.

Figure 4 (left) for T_a of 30°C: the 40 μg of capsaicin injection stimulated the activation of bar-pressing in 9 rats out of 10. After capsaicin injection, mean bar-pressing time rose from 23.7±3 sec to 108.5±18.7 sec. The effect of capsaicin is statistically significant (Mann and Whitney: U=1; $p<0.002$). In Fig. 4 (right) Tween 80 stimulated the activation of bar-pressing in 2 rats out of 10. Mean bar-pressing in the 10 rats 30 min before Tween 80 was 33.7±5.5 sec and 27.8±4 sec 30 min after.

Figure 5 compares the mean general time spent in bar-pressing before and after capsaicin or vehicle injection.

DISCUSSION

The present study is the first investigation of the effects on temperature regulation of capsaicin injected into the spinal subarachnoid space. Intrathecal capsaicin injection elicited a fall in body temperature with a rise in tail skin temperatures due to skin vasodilation. The latter would increase cutaneous heat loss, and so may at least partly account for the fall in T_{re}. Similar results have been obtained when cap-

saicin was injected subcutaneously, intravenously or intracerebrally. These results have been interpreted as an activation of heat-loss mechanisms [7, 12, 15].

Furthermore, the present results show that IP capsaicin injection did not raise T_{sp}. On the contrary, T_{sp} fell by 3.9°C. This was similar to the fall in hypothalamic temperature when capsaicin was injected cerebrally [7]. In the experiment of Dib, 1983 [8], IP capsaicin injection produced a fall in T_{re} that was similar to the IT capsaicin injection. It may be therefore concluded that peripheral and central capsaicin injection had the same effects on body temperature. IP or IT vehicle control (Tween 80) had no effect on body temperature.

Behavioral heat defense responses were enhanced by pressing the lever in order to obtain cool air after IT capsaicin injection. This confirms our previous results obtained after intracerebroventricular (ICV) injection of capsaicin [7]. This effect is specific for capsaicin, since under identical conditions vehicle of capsaicin did not activate bar-pressing much. But it is important to note that IT Tween 80 stimulated the activation of bar-pressing in 2 rats out of 10 at T_a of 30°C. The 8 rats that did not increase the activation of bar-pressing were again tested for IT capsaicin injection in order to check that the acute IT capsaicin induced the activation of bar-pressing behavior. In these rats bar-pressing behavior was enhanced significantly. In other experiments IT isotonic saline sometimes stimulated the activation of bar-pressing behavior (personal observation). It is possible that the activation of bar-pressing obtained by Tween 80 injection was due to an artefact. We have observed during the fanning behavior by bar-pressing after IT capsaicin injection, that T_s fell at T_a 30°C (Fig. 4 left). This fall in T_s was less important at T_a 20°C than at 30°C. The fall in T_s may depend on the use of bar-pressing. Because animals sometimes press the lever with their front paws, the head receive the cool air and little change occurred in T_s (Fig. 3 left). The animals sometimes pressed the lever with their rear paws or with their tails; under these conditions, the tail and the caudal part of the body receive the cool air and produce a fall in T_s (Fig. 4).

In the present experiment, some rats did not press the lever after capsaicin injection. This was much more obvious at the T_a of 20°C than at the T_a of 30°C. At the T_a of 20°C the rats perhaps did not need to press the lever much. The absence of bar-pressing is perhaps related to the position of the tip of the cannula. Besides the operant behavior, the rats perhaps used a different means of eliminating or dissipating heat-loss, such as body extension. The T_s rose in the rats that did not activate the behavioral regulation but, instead, activated autonomic thermoregulation. Since it is well known that there is a complementary relationship between behavioral and autonomic thermoregulation.

There is sound evidence that the thoraco-lumbar cord contains thermodetecor neurones similar to those located in the anterior-hypothalamic region [20]. Thermal information from spinal thermosensitive structures is transmitted to supraspinal sites by the spinothalamic tract [21]. Thus thermodetector units probably synapse with dorsal horn interneurones. It is clear that capsaicin in Tween 80 has very characteristic effects on autonomic and thermoregulatory behavior. At present, some uncertainty still persists regarding the site and mode of actions of capsaicin. For this reason, it must by hypothesized that:

(1) IT capsaicin acts directly on thermoregulatory heat-loss responses using the spinothalamic pathways.

(2) IT capsaicin acts on thermoregulation by directly exciting the hypothalamus warm-sensitive neurons which was absorbed in circulation from subarachnoid space. In fact, capsaicin quickly enters the brain from peripheral blood and has been found in high concentration in the brain 3 min and 10 min after intravenous and subcutaneous injection respectively [19].

(3) IT capsaicin acts on thermoregulatory behavior, in part by the release of substance P from primary afferent terminals. IT injection of capsaicin has been shown to deplete SP from terminals in the spinal cord [24]. Further, IT substance P injection stimulated the activation of bar-pressing behavior to obtain a cool air [11].

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