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Hyperthermia Induced in Rabbits by Organic Calcium Antagonists

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PALMI, M. AND G. P. SGARAGLI. *Hyperthermia induced in rabbits by organic calcium antagonists*. PHARMACOL BIOCHEM BEHAV **34**(2) 325–330, 1989.—Verapamil, nifedipine and cinnarizine, when injected intracerebroventricularly (ICV), induced a rise in core temperature related to the dose of the drug and accompanied by vasoconstriction of the ear vascular bed. On the contrary, the calcium channel activator BAY-K-8644, structurally related to nifedipine, elicited a dose-related hypothermic response which was accompanied by vasodilatation. The delay in onset of verapamil-induced hyperthermia was reduced by pretreating the animals with a dose of acetylsalicylic acid (ASA) which antagonized fever induced by *E. coli* endotoxin. BAY-K-8644 was shown to partially antagonize *E. coli* endotoxin-induced fever. These findings indicate that neurons responsible for temperature control are a target of organic calcium antagonists and suggest that calcium metabolism is of primary importance in the function of these cells.

Verapamil Cinnarizine Nifedipine BAY-K-8644 Acetylsalicylic acid *E. coli* endotoxin
ICV administration Rabbit Thermoregulation

REGULATION of cellular membrane activity in general and neuronal functions in the central nervous system are partially dependent upon the metabolism of calcium ions. In the last few years, evidence has been collected that calcium ions play an important role in the central mechanisms of thermoregulation. Experiments by Feldberg, Myers and their co-workers have shown that alterations in calcium concentrations in brain tissue have clear consequences on thermoregulation. The omission of Ca^{++} from the solution perfused from the lateral ventricles to the cisterna magna results in a rise in temperature in the unanesthetized cat or monkey (11,21). Furthermore, perfusion of the posterior hypothalamus with high Ca^{++} levels produces a sharp fall in body temperature accompanied by vasodilatation and reduced activity in the rabbit and cat (2,20).

Intense hyperthermia has also been observed when endogenous Na^{+} is artificially increased in the hypothalamus (2,20). These findings led to the hypothesis that the "set point" of the thermoregulatory system is determined by the balance between Ca^{++} and Na^{-} concentrations in cerebrospinal fluid (CSF). If this were the case, then drugs that alter calcium metabolism in the brain should affect Ca^{++} levels and upset the balance between Ca^{++} and Na^{+}. This, in turn, would lead to a shift in body temperature. To test this hypothesis we treated conscious rabbits intracerebroventricularly (ICV) with known calcium channel blockers and activators. These compounds inhibit and facilitate respectively, the intracellular inward calcium current, and possibly have opposite effects on body temperature. The data presented here showed that verapamil, nifedipine and cinnarizine evoked an hyperthermic response, whereas the calcium channel activator BAY-K-8644 induced hypothermia and partly antagonized the fever induced by bacterial endotoxin.

METHOD

Chemicals and Animals

Verapamil hydrochloride, nifedipine and cinnarizine were obtained from Sigma Chemical Co. (St. Louis, MO). BAY-K-8644 was a generous gift from Dr. G. Franckowiak (Bayer, Wuppertal, Germany). ASA was purchased from Farmitalia-Carlo Erba, Analytical Division (Milano, Italy) and *E. coli* endotoxin was obtained from Difco Laboratories (Detroit, MI). "Pyrogen-free" water was made from sterile water prepared for clinical use, passing it through a column (10 cm height; 1 cm, i.d.) packed with detoxi-gel (Pierce Chemical Co., IL). Aqueous solutions of the drugs to be administered were prepared shortly before the experimental sessions using sterilised volumetric devices and double distilled pyrogen-free water. ASA was dissolved in previously sterilised 0.1 N NaOH so that final pH was 7.0. Nifedipine and BAY-K-8644 were dissolved directly prior to use in a mixture of ethanol:H_2O (1:1 = v:v); both the preparation and the ICV administration of these latter compounds were performed in a dark chamber under a red light lamp. Cinnarizine was dissolved directly prior to use in a mixture of dimethylsulfoxide and ethylacetate (6:4 = v:v).

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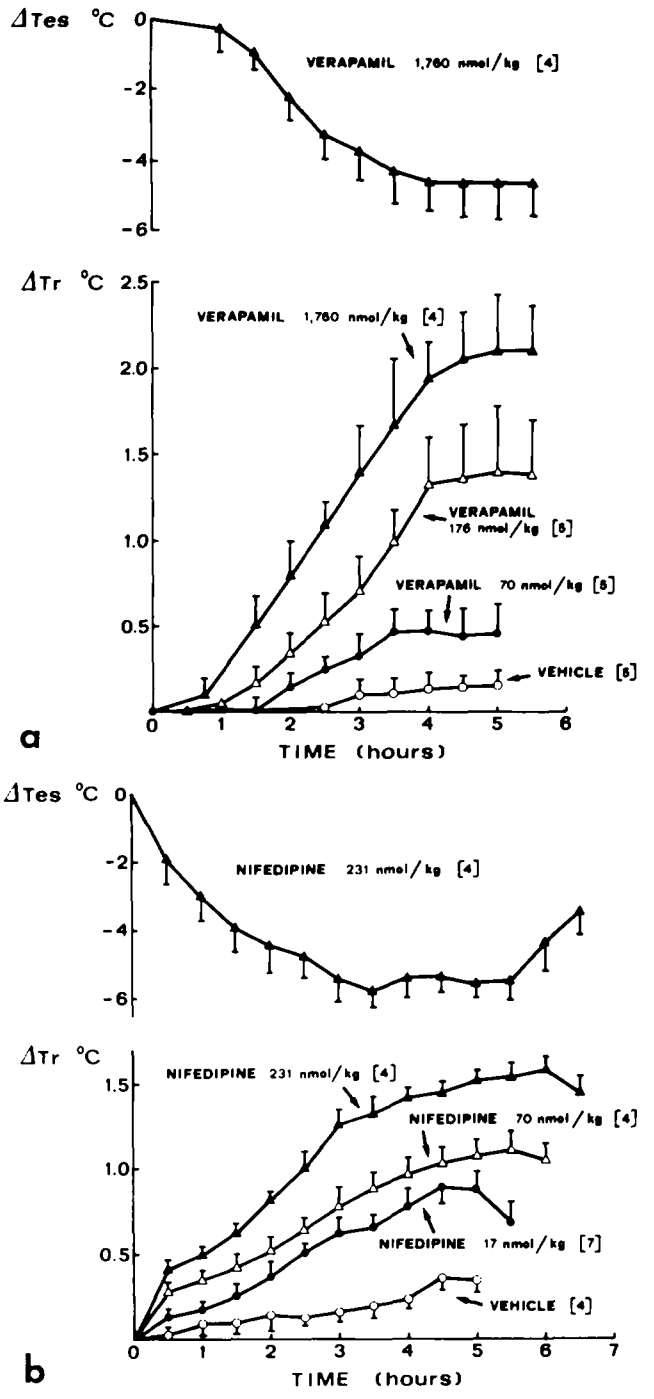

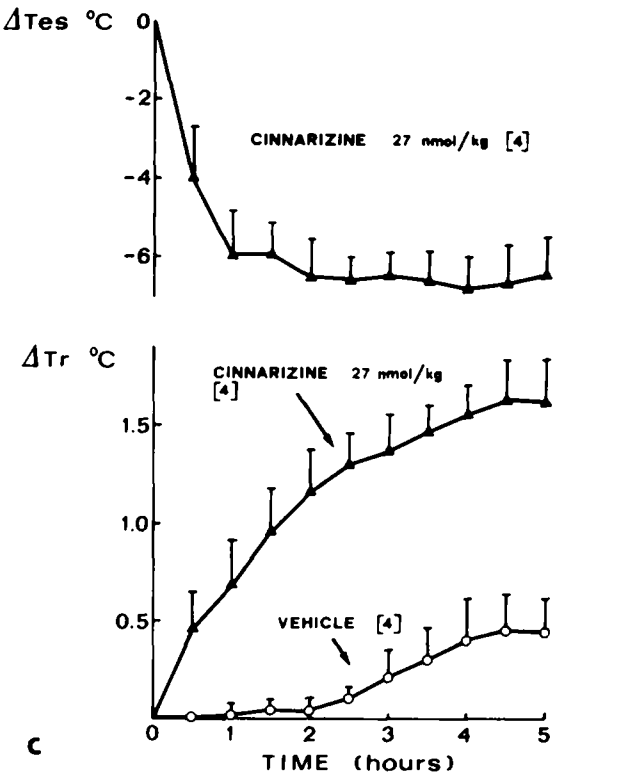

FIG. 1. Time course of the effects elicited by ICV injection of verapamil (A), nifedipine (B) and cinnarizine (C) on rectal (Tr) and ear skin (Tes) temperature of rabbits. Points are mean values and vertical bars represent S.E.M. Figures indicate the dose of the drug administered. The number of animals is given in parentheses. Linear regression analysis performed by variance analysis on the single values of ΔTr_{max} gave p values <0.008 (verapamil) and 0.01 (nifedipine). Tes was measured in animals treated with the highest dose. Vehicles alone [H_2O for verapamil; H_2O/ethyl alcohol mixture (1:1 = v:v) for nifedipine and dimethylsulfoxide/ethylacetate mixture (6:4 = v:v) for cinnarizine] were injected at the same volume used for drugs (10 μl).

The volumes of the ICV-injected drugs (verapamil, nifedipine, cinnarizine and BAY-K-8644) and their respective vehicles were the same (10 μl) and were delivered over a one-minute period by means of an Agla Micrometer Syringe (Burroughs Wellcome and Co., London, England).

Adult male New Zealand albino rabbits weighing 2.0 to 2.5 kg were used. Animals supplied with food and water ad lib were kept in individual cages at an ambient temperature (A.T.) of 20–23°C with a normal night and day cycle. All experiments were performed at constant chamber temperature (20°C) (Soc. KW, Siena, Italy).

Implantation of Inflow Cannula

Under sodium pentobarbitone (Nembutal) anaesthesia (IV, 30 mg/kg body weight), an inflow cannula guide was placed stereotaxically into a lateral cerebroventricle according to the coordinates indicated by Sawyer and co-workers (24), as previously described (26).

Selection of Animals for This Study

Animals with chronically-implanted ICV cannulas often respond with prolonged hyperthermia to the introduction of aqueous solutions into the cerebrospinal fluid spaces (11,12). In some animals, under the present experimental conditions, hyperthermia was elicited by simply injecting as little as 5 μl pyrogen-free water into the ventricles. This effect had a latency of about two hours and lasted more than 12 hours. Only animals which did not present

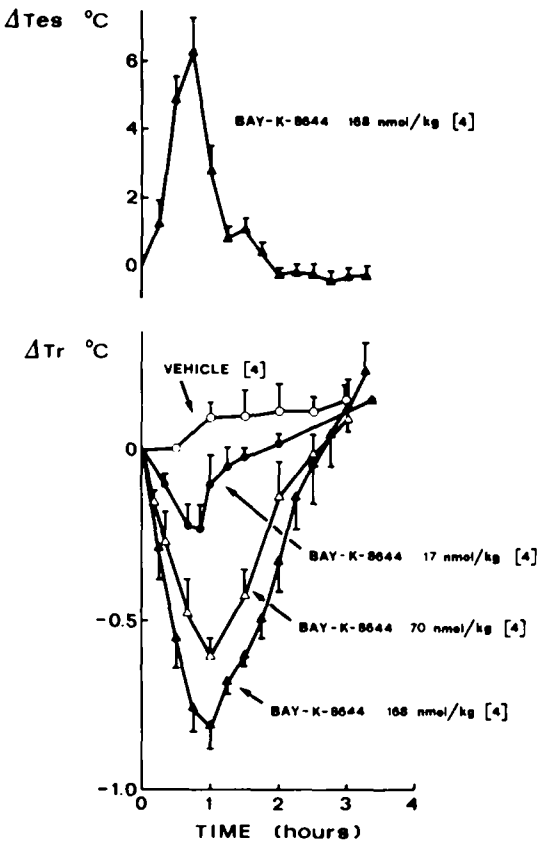

FIG. 2. Time course of the effects elicited by BAY-K-8644 on rectal (Tr) and ear skin (Tes) temperature of rabbits. Points are mean values and vertical bars represent the S.E.M. Figures mean the dose of the drug administered. The number of animals is given in parentheses. Linear regression analysis performed by variance analysis on the single values of ΔTr_{max} gave a p value of 0.006. Tes was measured in animals treated with the highest dose. Vehicle alone (H_2O/ethyl alcohol mixture; 1:1 = v:v) was injected at the same volume used for the drug (10 μl).

this hyperthermic response were used in subsequent experiments. After each experimental session, the selected rabbits were again tested intracerebroventricularly with pyrogen-free water. Animals which became hyperthermic were rejected and the results previously obtained from them discarded.

Experimental Procedure

Effect on rectal temperature of verapamil, nifedipine, cinnarizine and BAY-K-8644. Core and ear skin temperatures were recorded using a T.E. thermometer (Ellab Instruments, Copenhagen, Denmark). Core temperature was measured inserting a thermocouple probe 10 cm into the rectum. Temperature was monitored for at least 1 hr before any drug was injected. Different groups of animals were used to test the effect of each drug. Five rabbits were used to test verapamil-induced hyperthermia; each was given three doses of the drug (70, 176 and 1,760 nmol/kg corresponding to 31.8, 80, and 800 μg/kg respectively) at 72-hr intervals. Seven animals received three doses of nifedipine (17, 70, and 231 nmol/kg corresponding to 5.88, 24.2 and 79.9 μg/kg, respectively), again at 72-hr intervals, whereas four rabbits were given a single dose of 27 nmol/kg (9.95 μg/kg) of cinnarizine. Four rabbits were used to test BAY-K-8644-induced hypothermia; each received three doses of the drug (17, 70 and 168 nmol/kg corresponding to 6.05, 24.9 and 59.8 μg/kg respectively) at 72-hr intervals.

Effect of ASA on E. coli *endotoxin- and verapamil-induced hyperthermia.* One series of experiments was performed to check the capacity of ASA (80 mg/kg IV) to antagonize the pyrogenic effect of *E. coli* endotoxin (0.1 μg in 1 ml sterile saline, given IV). Five animals underwent four experimental sessions at 24-hr intervals. In the first session, bacterial endotoxin alone was injected, while in the second, the endotoxin injection was preceded 30 min earlier by ASA. In the third and fourth sessions, endotoxin alone and ASA alone, respectively, were administered. In another series of experiments, the effect of ASA on verapamil-induced hyperthermia was studied. Five rabbits were used in three experimental sessions, at 48-hr intervals. In the first session, verapamil alone (90 nmol/kg corresponding to 40.9 μg/kg) was given ICV; in the second session, the same dose of verapamil was injected 30 min after IV administration of ASA (80 mg/kg). In the third session, animals received ICV double-distilled pyrogen-free sterile water 30 min after ASA administration.

Effect of BAY-K-8644 on fever induced by E. coli *endotoxin.* Four animals were tested with IV *E. coli* endotoxin (0.1 μg) on

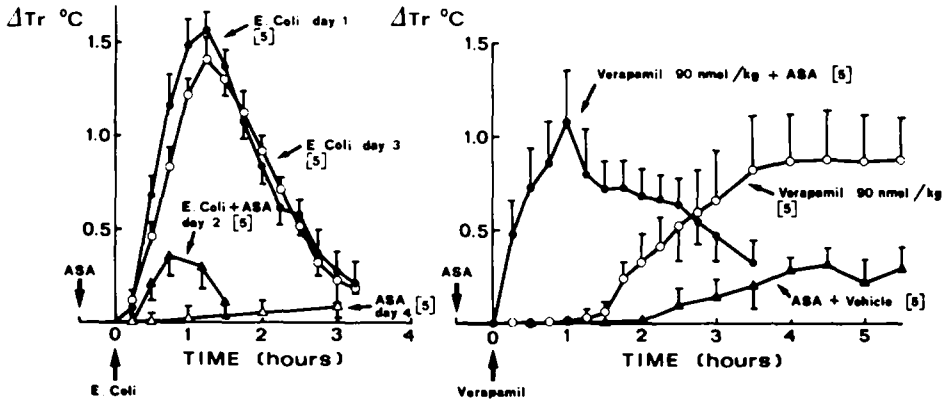

FIG. 3. Effect of ASA on *E. coli* endotoxin-induced fever (left panel) and on the hyperthermic response to verapamil (right panel). The two experiments were performed separately on two groups of five animals. *E. coli* endotoxin and ASA were injected IV at a dose of 0.1 μg and 80 mg/kg, respectively. Vehicle (double distilled, "pyrogen-free" and sterile water) was injected ICV 30 min after IV injection of ASA. The arrows on the abscissa indicate the time of injections. Points are mean values and vertical bars represent the S.E.M.

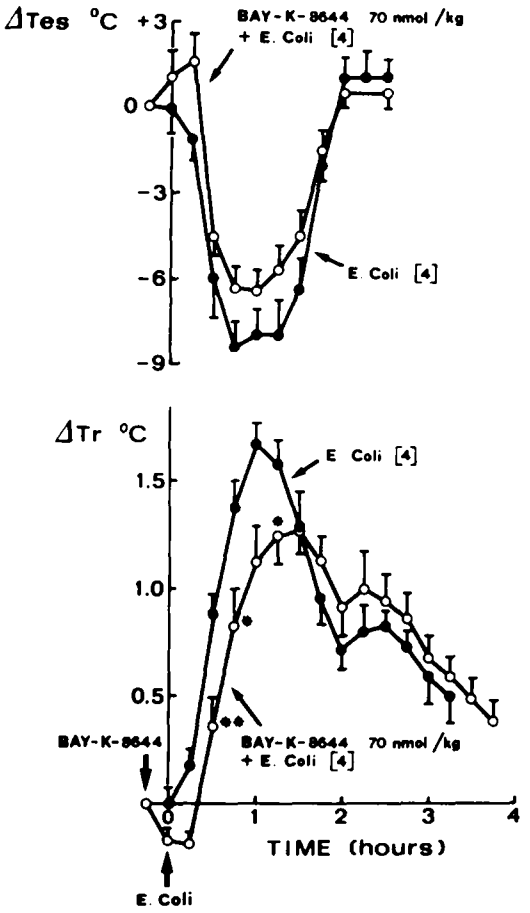

FIG. 4. Effect of BAY-K-8644 on *E. coli* endotoxin-induced fever as evidenced by the measurement of rectal (Tr) and ear skin (Tes) temperature of rabbits. One group of four rabbits was injected IV with a dose of 0.1 μg of *E. coli* endotoxin every day for two subsequent days. The first day the endotoxin injection was preceded by 15 min by ICV injection of BAY-K-8644. The arrows on the abscissa indicate the time of injections. Points are mean values and vertical bars represent the S.E.M. Values with asterisks are significantly different from those observed at the same time after *E. coli* injection when animals had not been pretreated with BAY-K-8644. (*$p<0.05$; **$p<0.02$; by analysis of variance for paired data.)

two consecutive days. The first day the endotoxin injection was preceded 15 min earlier by ICV injection of BAY-K-8644 (70 nmol/kg corresponding to 24.9 μg/kg). None of the above treatments caused death in the animals. The few animals whose core temperature did not return to normal after 24 or 72 hr following treatment were eliminated from the study.

Statistical Analyses

Linear regression analysis was performed by variance analysis on the maximal changes in rectal temperature against drug doses. Student's *t*-test and variance analysis were performed. The level of significance was set at $p<0.05$.

RESULTS

Effect on Temperature of Verapamil, Nifedipine, Cinnarizine and BAY-K-8644

As shown in Fig. 1, the ICV injection of verapamil (panel A), nifedipine (panel B) or cinnarizine (panel C) produced a dose-dependent rise in core temperature accompanied by vasoconstriction of the ear vascular bed. The hyperthermia induced by verapamil had a latency of 1–2 hr, whereas that elicited by nifedipine and cinnarizine was characterized by a prompt onset. Replacement of the nitro group of nifedipine by a CF_3 group converts this blocker into the calcium channel activator designated BAY-K-8644. ICV injection of this compound elicited prompt dose-related hypothermia accompanied by vasodilatation of the ear vascular bed (Fig. 2). Gross neurological behaviour was different after each compound. While the animals tested with organic calcium antagonists exhibited normal neurological behaviour, those treated with BAY-K-8644 were sedated and sleepy.

Effect of ASA on E-coli *Endotoxin- and Verapamil-Induced Hyperthermia*

As shown in Fig. 3, left panel, the IV administration of *E. coli* endotoxin elicited fever that started almost immediately after administration, peaked within 75–90 min (ranges of maximal changes in rectal temperature were 1.3–1.5°C) and returned to basal values within 3 hr. As shown in the same panel, the IV administration of ASA that did not affect normal body temperature markedly reduced fever induced by bacterial endotoxin. In contrast, pretreatment with ASA (right panel) hastened the hyperthermic response to verapamil.

Effect of BAY-K-8644 on Fever-Induced by E. coli *Endotoxin*

As shown in Fig. 4, BAY-K-8644 partially antagonized *E. coli* endotoxin-induced fever by delaying the onset and peak of the hyperthermic response. On the other hand, BAY-K-8644 did not seem to affect *E. coli*-induced vasoconstriction of the ear vascular bed.

DISCUSSION

The present results show that verapamil, nifedipine and cinnarizine, injected ICV into rabbits, induce a dose-related increase in body temperature. If we assume that once injected into the cerebroventricular space these compounds are evenly distributed throughout the brain, then the concentrations at which they are active would be of about one order of magnitude higher than those effective in in vitro experiments which have clearly established their calcium antagonist properties (13, 18, 28). The calcium channel activator BAY-K-8644 (25,29) elicited the opposite effect in a dose-related manner. The animals became sedated and sleepy. This agrees with the finding of ataxia and decreased motor activity in rats injected IP with this compound (5). The suppression of behavioural activity with sleepiness and catalepsy has also been reported in cats when the hypothalamic region of the animals was perfused with high calcium level containing solutions (30). Direct evidence of calcium involvement in thermoregulation has been provided by experiments in which the perfusion of excess calcium in the extracellular fluid of the hypothalamus was found to greatly reduce the rise in body temperature induced in animals by physical exercises (14, 15, 22). A linear relationship between calcium excess in the hypothalamus or lateral ventricles and the decrease in body temperature was also found in various mammals kept at rest at ambient temperature (16). It has recently been found that different calcium channel antagonists of the dihydropyridine (DHP) type, given per os, reduce the hypothermic effect of apomorphine SC administered in rodents, thus providing an indirect evidence of calcium role in thermoregulation (8). Finally, taurine, which is known to influence the calcium metabolism of excitable tissues (17,31), induces marked hypothermia (26) and antagonizes the hyperthermia induced by verapamil (27) when

administered ICV in mammals. All these findings support the view that the pharmacological property shared by these compounds, i.e., calcium channel blocking or facilitating activity, may account for their effects on body temperature. This would provide further evidence in favour of the theory of Feldberg and Myers (11,21) that calcium is involved in the regulation of the thermal set-point in the posterior hypothalamus. They suggest that the set-point for body temperature is determined by sodium-calcium balance. In the present study we have shown that when this balance is upset by drugs that modify calcium metabolism, the animal responds with hyperthermia or hypothermia, according to the calcium channel blocking or activating properties respectively, of the drug. It is not yet possible to indicate whether the posterior hypothalmus is the area sensitive to the variations in calcium metabolism induced by these drugs; even though our study does not exclude this possibility, experiments with localized drug administration are required to clarify this point. The present findings, however, argue against the possibility that prostaglandin synthesis is involved in calcium antagonist-induced hyperthermia. ASA, in fact, not only failed to counteract the verapamil effect, but seemed to facilitate the action of the drug on body temperature by reducing the lag phase which characterizes the hyperthermic response to this drug.

This effect is difficult to explain. Since salicylate has been shown to have a direct effect on membrane permeability to ions in molluscan neurons (1, 6, 19), we can only cautiously invoke a similar mechanism to explain our results with ASA in mammals.

It has been established that bacterial endotoxin and other agents induce the release of interleukin 1 (IL-1) (9,10), which is highly suspected of inducing fever through the activation of prostaglandin synthesis (3, 7, 32). Although the exact mechanism by which IL-1 elicits fever is not yet clarified, one of the most widely accepted hypotheses is that IL-1 acts by increasing intracellular concentrations of calcium, thus activating the enzymes which lead to prostaglandin synthesis and fever (4, 10, 23). However, our findings that the calcium-channel blockers induce hyperthermia, whereas the calcium-channel activator BAY-K-8644 induces hypothermia and antagonizes the fever induced by *E. coli* endotoxin, are at variance with the interleukin-1 data. We can therefore postulate the existence of two different cellular target sites responsible for the increase in body temperature when challenged by organic calcium antagonist or by *E. coli* endotoxin.

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