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THE ANTIPYRETIC EFFECT OF TILORONE HYDROCHLORIDE IN THE CAT

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1 The antipyretic activity of tilorone hydrochloride was studied in conscious, unrestrained cats provided with implanted jugular venous catheters, third cerebral ventricular (i.c.v.) cannulae and retroperitoneal thermocouples.

2 In afebrile animals, 10 mg/kg i.v. or 1 mg i.c.v. tilorone hydrochloride did not alter body temperature, but 20 mg/kg i.v. or 2 to 5 mg i.c.v. caused hypothermia and various behavioural responses.

3 Non-hypothermogenic doses of tilorone (i.v. or i.c.v.) antagonized hyperthermic responses to leucocytic pyrogen (i.v. or i.c.v.), bacterial pyrogen (i.c.v.) and sodium arachidonate (i.c.v.) but did not antagonize prostaglandin E_1 (i.c.v.).

4 These results indicate that tilorone has an antipyretic action within the central nervous system that is distinct from its hypothermogenic action. Although there is no published evidence to indicate that tilorone can inhibit prostaglandin synthesis peripherally, its ability to reduce hyperthermic responses to arachidonate suggests that it can inhibit prostaglandin synthesis within the brain.

Introduction

Antipyretics do not necessarily act, as has been proposed (Vane, 1971), via inhibition of prostaglandin synthetase. Paracetamol and indomethacin have been shown to be more potent in reducing febrile responses to pyrogens than in antagonizing the hyperthermic response to arachidonate (Clark & Cumby, 1976), the precursor of prostaglandin E_2. This suggests that, if prostaglandins mediate pyrogen-induced fevers, these antipyretics exert a major action at some step before prostaglandin synthesis. Although there is considerable evidence in the literature (Coceani, 1974; Veale & Cooper, 1974; Milton, 1976) compatible with involvement of a prostaglandin of the E series in pyrogen-induced fevers, a number of recent reports, on the contrary, indicate that prostaglandins are not necessarily involved in pyrogenic responses. Animals that do not develop hyperthermia after prostaglandins may still respond to pyrogen injections with fever (Baird, Hales & Lang, 1974; Pittman, Veale & Cooper, 1975; Veale & Cooper, 1975). An increase in cerebrospinal fluid (CSF) prostaglandin levels is apparently not essential for pyrogen-induced fevers (Cranston, Hellon & Mitchell, 1975b), and hyperthermic responses to prostaglandin E_2 can be reduced by central administration of the prostaglandin antagonists SC 19220 and HR 546 without altering febrile responses to leucocytic pyrogen (Cranston, Duff, Hellon, Mitchell & Townsend, 1976).

Tilorone has been reported to share with non-steroidal anti-inflammatory agents, such as aspirin, indomethacin and phenylbutazone, the ability to inhibit leucocyte migration into sponges implanted subdermally into rats (Ford-Hutchinson, Bolam & Walker, 1976). Unlike these other agents, tilorone had no effect on prostaglandin content of the sponge exudates or on prostaglandin synthesis by guinea-pig lung enzyme. These authors proposed tilorone as representative of a new class of anti-inflammatory drugs which act by a mechanism other than inhibition of prostaglandin synthetase. We studied tilorone first to determine if it had an antipyretic action and, when it did, to see if it antagonized arachidonate-induced hyperthermia as an indication of its ability to inhibit prostaglandin synthesis within the central nervous system (CNS).

Methods

Eighteen adult mongrel cats of mean weight 3.1 kg were used. Procedures for care and feeding of the animals, for recording body temperature chronically

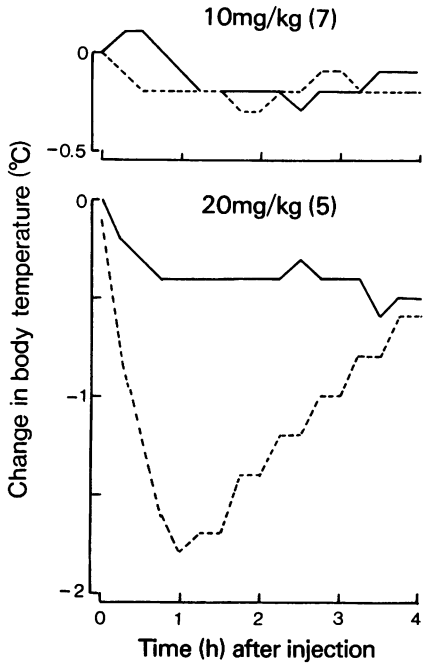

Figure 1 Cross-over comparisons of the mean effects of tilorone hydrochloride (dashed line) and saline (complete line) given i.v. on body temperature of afebrile cats. The numbers in parentheses indicate the number of animals used.

from the retroperitoneal space, for implanting i.v. catheters or third cerebral ventricular cannulae, for sterilization of glassware and for otherwise avoiding pyrogenic contamination have been described previously (McCarthy & Borison, 1966; Clark & Moyer, 1972). Environmental temperature was maintained at 22 ± 1°C. Unless otherwise indicated, the average of body temperature readings at 30, 15 and 0 min before the first injection was used as the base line from which changes were measured. Unless otherwise specified, cross-over designs were used in which each animal received each of the tests in randomized order. In any given set of cross-over experiments, injections were given at specific times to avoid errors due to diurnal temperature variations. Intravenous injections were flushed in with 1.0 ml 0.9% w/v NaCl solution (saline). Intracerebroventricular (i.c.v.) injections were given in a volume of 0.05 ml, and the cannulae were flushed with 0.1 ml saline between tests.

Deviation of body temperature from base line was tabulated at 15 min intervals, and changes in temperature were quantified as a 'thermal response index' (TRI; Clark, 1970), one unit of which is equivalent to a 1°C change lasting for 1 h (Clark & Cumby, 1975). TRIs were determined from the time of the final injection for the number of hours indicated by a subscript. Results were analyzed by the paired *t* test.

Stock solutions of tilorone [2,7-bis(diethylaminoethoxy)fluoren-9-one] hydrochloride (Merrell-National Laboratories) and *Salmonella typhosa* endotoxin (Difco) in saline were stored at 4°C. Doses of tilorone refer to the hydrochloride. Saline solutions of sodium arachidonate (Nu-Chek-Prep, Inc., Elysian, Minnesota) were kept frozen at −9°C and were thawed as necessary to obtain portions for injections (Clark & Cumby, 1976). Leucocytic pyrogen and prostaglandin E_1 (Upjohn) were prepared and stored respectively as described previously (Clark & Cumby, 1975). Endotoxin, arachidonate and prostaglandin were injected i.c.v. Tilorone and leucocytic pyrogen were administered both i.v. and i.c.v.

Results

Hypothermic effect of tilorone

A dose of 10 mg/kg tilorone injected i.v. did not significantly alter body temperature in afebrile cats (Figure 1, Table 1) or cause obvious side effects. A dose of 20 mg/kg, however, caused a significant reduction in temperature accompanied by tachypnoea,

Table 1 The hypothermic effect of tilorone hydrochloride injected i.v. in afebrile cats

Dose (mg/kg)	n	*Maximum decrease (°C) (mean* ± s.e. mean)	*TRI_4 (Δ°C × h)* (mean ± s.e. mean)
0	7	0.4 ± 0.1	−0.5 ± 0.4
10		0.5 ± 0.1	−0.8 ± 0.2
0	5	0.7 ± 0.1	−1.6 ± 0.3
20		1.9 ± 0.4*	−4.9 ± 1.2*

TRI = Thermal response index; see Methods section.
* Significantly different from responses to saline vehicle designated as dose = 0 ($P < 0.05$).

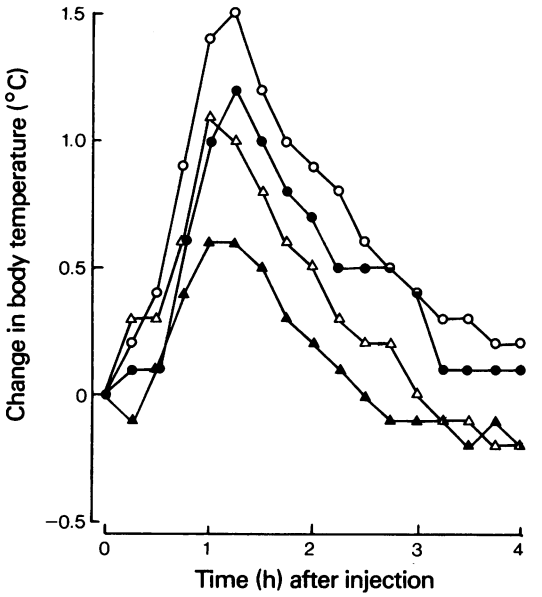

Figure 2 Reduction in pyrogenic responses to leucocytic pyrogen produced by various doses of tilorone hydrochloride: (●) 2.5 mg/kg; (△) 5 mg/kg; (▲) 10 mg/kg; (○) Control (saline). Both agents were given i.v. Preliminary assays of pyrogen were performed with each cat to determine a dose which elevated body temperature about 1.5°C. This volume was then given to that cat in each test in the cross-over. Tilorone or saline solution was given immediately after the pyrogen. Mean responses of seven cats.

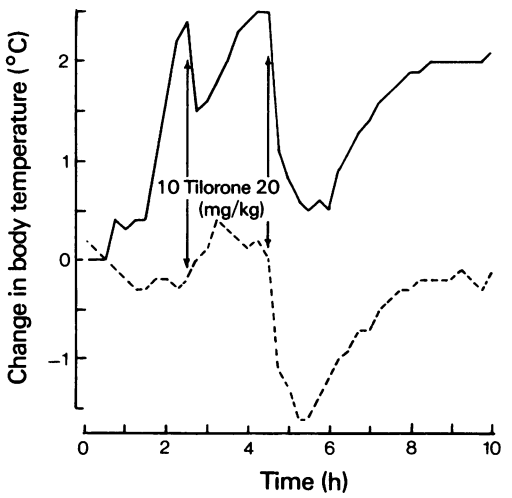

Figure 3 Interruption by tilorone of the pyrogenic response to leucocytic pyrogen (complete line) injected i.c.v. in a cat given two doses of tilorone i.v. at the times indicated by the arrows. Pyrogen (first test) or saline (second test; dashed line) was injected at time zero.

mydriasis and by vocalization or hissing. Ataxia and defaecation were also exhibited by some of the animals. Injected i.c.v. in four cats, 2 to 5 mg doses caused decreases in temperature of 0.6 to 2.2°C and behavioural changes similar to those seen after i.v. inspection of 20 mg/kg. Two of these animals also vomited within 10 minutes. Lower doses of tilorone i.c.v. caused little or no change in body temperature or behaviour.

Antipyretic effect of tilorone

Non-hypothermogenic doses of tilorone produced a dose-related reduction in the pyrogenic response to leucocytic pyrogen when both were given i.v. (Figure 2, Table 2). Tilorone (10 mg/kg i.v.) also effectively interrupted responses to central administration of leucocytic pyrogen in two animals (Figure 3). Note that in the example given, in a subsequent test with saline,

Table 2 Reduction of leucocytic pyrogen-induced fever by tilorone hydrochloride in seven cats. Both agents were given i.v.

Tilorone dose (mg/kg)	*Maximum increase (°C)* (*mean* ± s.e. mean)	TRI_4 (Δ°C × *h*) (mean ± s.e. mean)
0	1.6 ± 0.1	2.7 ± 0.5
2.5	1.3 ± 0.1	2.0 ± 0.3
5	1.3 ± 0.1	1.3 ± 0.4*
10	0.8 ± 0.2*	0.4 ± 0.7**

TRI = Thermal response index; see Methods section.
* Significantly different from response to pyrogen + vehicle ($P < 0.025$); ** $P < 0.005$.

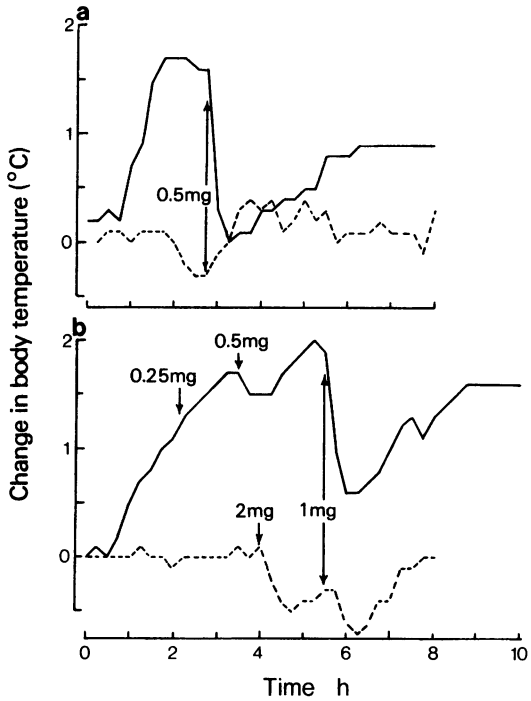

Figure 4 Examples of antipyretic and hypothermic responses to tilorone injected i.c.v. In (a) a marked antipyretic effect was produced by a dose of tilorone which did not cause hypothermia. (b) Illustrates a dose-related antipyretic effect of tilorone, which was considerably greater than the hypothermic effect in a subsequent test. Complete line: leucocytic pyrogen; dashed line: saline.

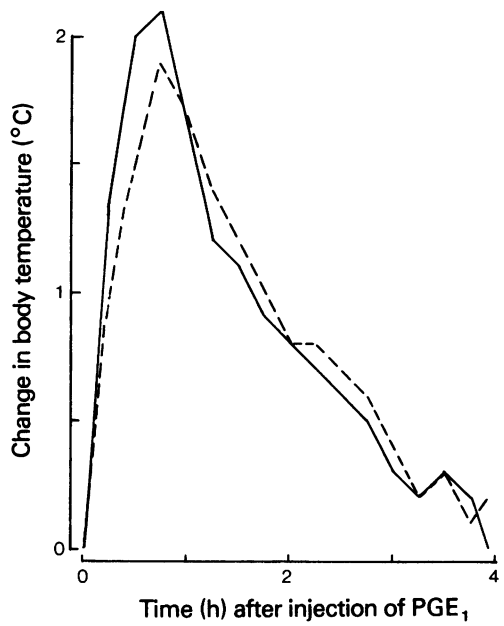

Figure 5 Tilorone (10 mg/kg, dashed line) or saline (complete line) was given i.v. 30 min before i.c.v. administration of prostaglandin E_1 (PGE_1) 0.3 μg. Mean responses of four cats.

10 mg/kg tilorone did not cause hypothermia while the 20 mg/kg dose did. When both agents were injected i.c.v. in seven tests. tilorone consistently interrupted pyrogen-induced fevers (Figure 4).

Lack of antagonism of prostaglandin by tilorone

When tilorone was given i.v. 30 min before i.c.v. administration of prostaglandin E_1, it did not appreciably alter the hyperthermic response (Figure 5, Table 3).

Comparison of the ability of tilorone to antagonize bacterial endotoxin and sodium arachidonate

Because hyperthermia develops more slowly after i.c.v. injection of endotoxin than after arachidonate (Clark & Cumby, 1976), endotoxin was given at 10 h 00 min and arachidonate at 12 h 00 min. Tilorone or saline was injected i.v. at 14 h 00 min when mean elevations of body temperature ranged from 1.6 to 2.0°C. Responses to both hyperthermogenic agents were reduced after tilorone (Figure 6, Table 4). To facilitate comparison of the effects of tilorone on responses to

Table 3 Lack of antagonism of prostaglandin E_1 injected i.c.v. by tilorone hydrochloride injected i.v. in four cats

Tilorone dose (mg/kg)	*Maximum increase (°C)* (mean ± s.e. mean)	TRI_4 ($\Delta°C \times h$) (mean ± s.e. mean)
0	2.2 ± 0.2	3.5 ± 1.3
10	1.9 ± 0.2	3.5 ± 0.6

TRI = Thermal response index; see Methods section.

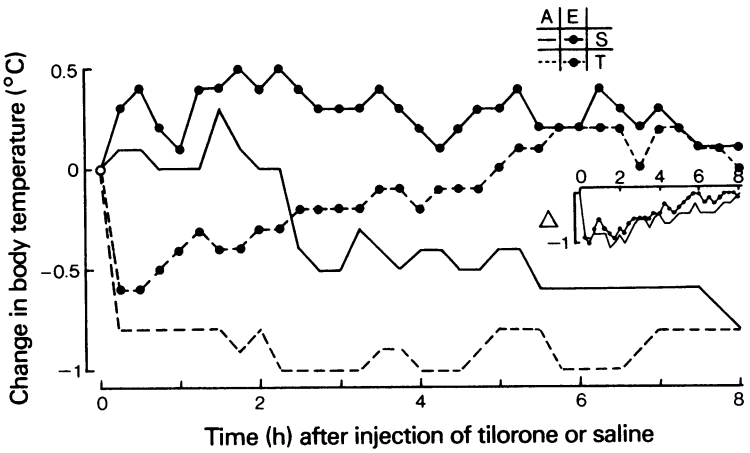

Figure 6 Comparison of changes in hyperthermic responses to i.c.v. injections of sodium arachidonate (A, 200 μg) and endotoxin (E, 0.1 g) after i.v. injection of tilorone (10 mg/kg). Mean changes in temperature from hyperthermic levels at the time of tilorone (T) or saline (S) injection in four cats. In the inset, the (●——●) line shows the difference between the mean responses to endotoxin after saline and after tilorone (line E–S minus line E–T) while the complete line likewise shows the difference between the mean responses to arachidonate (line (A–S minus line A–T).

endotoxin and arachidonate, the differences between the appropriate control (saline) curves and the test (tilorone) curves are shown in an inset. Tilorone antagonized both agents approximately equally. However, there was a negative correlation ($r = -0.71$, $P < 0.2$) between the amounts of inhibition of the responses to the two hyperthermogenic agents.

Discussion

Tilorone was chosen for this study because it does not inhibit prostaglandin synthesis, at least in some systems in the periphery (Ford-Hutchinson *et al.*, 1976). Antipyretic doses of paracetamol and perhaps indomethacin apparently decrease prostaglandin synthesis in the CNS *in vivo*, as indicated by a reduction in the hyperthermic response to central injection of arachidonate (Clark & Cumby, 1976). However, these antipyretics were considerably more potent in inhibiting febrile responses to bacterial endotoxin and leucocytic pyrogen. A demonstration that tilorone had no effect on arachidonate-induced hyperthermia but still antagonized pyrogens would have provided even stronger evidence that antipyretics can produce their effect without inhibiting prostaglandin synthetase. Like other anti-inflammatory agents, tilorone did exhibit antipyretic activity, but it also antagonized arachidonate, indicating that tilorone can inhibit prostaglandin synthesis in the CNS. In this regard it resembles paracetamol which has very little ability to inhibit prostaglandin synthetase peripherally but is effective in brain tissue (Flower & Vane, 1972). Nevertheless, such inhibition may not account for the antipyretic effect of tilorone since the degrees of antagonism of arachidonate and endotoxin correlated negatively. Likewise it is unlikely that the hypothermic action of tilorone is due to reduced prostaglandin synthesis since prostaglandins do not appear to be involved in maintenance of normal body temperature (Cranston, Hellon & Mitchell, 1975a; Pittman, Veale & Cooper, 1976; Cammock, Dascombe & Milton, 1976).

Tilorone inhibited responses to both peripherally and centrally injected pyrogens. Hence, its antipyretic

Table 4 Reduction of i.c.v. endotoxin- and arachidonate-induced hyperthermias by tilorone hydrochloride injected i.v. in four cats

	TRI_5 (Δ°C × h) Pretreatment	
Tilorone dose (mg/kg)	*Endotoxin (0.1 μg)*	*Sodium arachidonate (200 μg)*
0	1.5 ± 1.5	−1.0 ± 1.0
10	−1.4 ± 1.0*	−4.4 ± 1.3*
Δ	2.9	3.4

TRI = thermal response index; see Methods section. Values are mean ± s.e. mean.
* Significantly different from response to vehicle ($P < 0.05$).

activity does not require that it block entry of leucocytic pyrogen into the CNS. Tilorone was also effective when small doses were given i.c.v., so it can exert its action centrally. In these respects it is similar to other antipyretics which have been studied in our laboratory (Clark, 1970; Clark & Moyer, 1972; Clark & Coldwell, 1972; Clark & Cumby, 1975). By comparison with data from these previous studies, 5 to 10 mg/kg tilorone is roughly equivalent in antipyretic activity to 20 to 40 mg/kg sodium salicylate, 5 to 10 mg/kg paracetamol and 10 to 20 μg/kg indomethacin. The inability of tilorone to antagonize prostaglandin-induced hyperthermia is also similar to other antipyretics (Milton & Wendlandt, 1971; Clark & Cumby, 1975).

Doubling the dose of tilorone from 10 to 20 mg/kg i.v. (or from 1 to 2 mg i.c.v.) caused hypothermia. Hypothermia was not a passive response due to general impairment of thermoregulatory function since it was accompanied and at least partially produced by tachypnoea. Whether these responses were due to an action on the thermoregulatory system such as to decrease set-point or to a more direct stimulation of respiratory centres was not studied. Shivering was not seen to oppose the fall in temperature, indicative perhaps of a reduced set-point. A variety of other effects were also evoked by hypothermic doses of tilorone. Thus, compared to other antipyretics, it appears to have a relatively narrow margin between antipyretic and toxic doses, which if similar in man might preclude its use clinically as an antipyretic. In the cat, for comparison, a good antipyretic effect is obtained with indomethacin at 40 μg/kg, and doses up to 1 to 2 mg/kg have been given without apparent toxicity (Clark & Cumby, 1975; Cranston *et al.*, 1975a). Paracetamol is antipyretic at 10 mg/kg (Clark & Moyer, 1972) and toxic at about 100 mg/kg (Finco, Duncan, Schall & Prasse, 1975), while salicylates are antipyretic at 40 mg/kg (Clark & Moyer, 1972) and evoke emesis and other signs of toxicity in some cats at 100 to 200 mg/kg (Larson, 1963; Clark, Montoya & Pomarantz, 1972).

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