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Antagonism of Hyperthermogenic Agents by L 8027, an Inhibitor of Prostaglandin and Thromboxane Synthetase (40473)

WESLEY G. CLARK AND J. M. LIPTON

Departments of Pharmacology, Physiology and Neurology, Southwestern Medical School, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235

Most nonsteroid anti-inflammatory agents used clinically are acidic, perhaps because they are more readily concentrated in inflamed tissue than chemically related alkaline compounds (1). On the other hand, L 8027 [3-(2-isopropyl indolyl)-3 pyridyl ketone] is a basic compound which has been reported to have potent antipyretic and anti-inflammatory activity in the rat (2), to inhibit both prostaglandin synthetase (3, 4) and thromboxane synthetase (4) and to abolish hypotensive responses of rabbits to sodium arachidonate (3). Another response to arachidonate, given intracerebroventricularly, is hyperthermia (5–8). This response is apparently not due entirely to synthesis of prostaglandin since pretreatment with the prostaglandin antagonists SC 19220 and HR 546 reduced but did not abolish the hyperthermia in the rabbit (7). Laburn *et al.* (7) concluded that an arachidonate derivative other than a prostaglandin, possibly a prostaglandin endoperoxide precursor or thromboxane, must also be hyperthermogenic. However, stable analogs of the endoperoxides, which are biologically active in some systems, did not cause hyperthermia in the rat (9) so it is likely that the natural endoperoxides must be converted further to active derivatives. The acidic antipyretics acetaminophen and indomethacin have been shown to inhibit pyrogen-induced fever to a considerably greater extent than arachidonate-induced hyperthermia (6), while tilorone, another basic anti-inflammatory agent, inhibited both hyperthermogenic agents about equally (10). The present study was performed to determine the relative sensitivities to blockade by L 8027 of hyperthermic responses to three agents, a bacterial pyrogen, sodium arachidonate and a prostaglandin.

Materials and methods. A total of eight cats weighing 3.0–4.8 kg were used. Procedures for care and feeding of the animals, for recording body temperature chronically from the retroperitoneal space, for implanting jugular venous catheters and third cerebral ventricular cannulas, for sterilization of glassware and for otherwise avoiding contamination by pyrogens have been described previously (11, 12). Environmental temperature was maintained at 22 ± 1°. Experimental designs were used in which each of six cats received each of the tests in randomly determined order.

The average of body temperature readings 0, 15 and 30 min before injection of L 8027 or ethanol control vehicle was used as the base line for determination of temperature changes. The mean base line temperature ± SE prior to experiments with L 8027 was 38.3 ± 0.1° and prior to experiments with vehicle was 38.5 ± 0.1°. Deviations of body temperature from base line were tabulated at 15-min intervals, and changes in temperature were quantified as a 'terminal response index' (TRI), one unit of which is equivalent to a 1° change lasting for 1 hr (13). TRIs were determined from the time of the final injection for the period of time indicated in the Tables. "Total" TRIs were determined until the change in response after administration of L 8027 equaled the change in temperature in the same cat after administration of ethanol in the appropriate control experiment.

Stock solutions of 1–4 mg/ml sodium arachidonate (Nu-Chek-Prep, Inc., Elysian, Minnesota) in 0.9% NaCl solution were kept frozen at −9°. These were thawed as necessary to obtain portions for injections and then refrozen. A stock solution of *Salmonella typhosa* endotoxin (Difco, 1 μg/ml) in saline solution was stored at 4°. Prostaglandin E_1 was stored at −9° in 95% ethanol. Shortly before use, the ethanol was evaporated by blowing nitrogen over the solution, and the prostaglandin residue was redissolved in saline solution. These agents were injected in-

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traventricularly in 0.05 ml, and the cannulas were flushed with 0.10 ml saline solution between tests. L 8027, dissolved in 95% ethanol shortly before use, was given iv in a volume of 0.10 ml/kg. To avoid precipitation of L 8027 inside the catheters, 0.05 ml ethanol was injected both before and after L 8027 and was followed immediately by a final flush with 1.0 ml saline solution. Preliminary experiments established that 1–2 mg/kg L 8027 interrupted febrile responses to endotoxin without causing obvious side effects or distress in the animals. In the experiments described below, the effect on body temperature of 2 mg/kg L 8027 was compared with the effect of ethanol vehicle alone.

Results. A very small but prolonged and consistent hypothermia developed after administration of L 8027 to afebrile cats (Fig. 1, Table Ia). Mean body temperature was lowered approximately 0.2° for 8 hr after L 8027 injection.

For comparison of the abilities of L 8027 to antagonize a pyrogen and arachidonate, endotoxin, which causes relatively slow development of fever, was given at 10:00 AM while arachidonate was given at noon. L 8027 or vehicle was given at 2:00 PM when mean increases in body temperature were over 1.8°. L 8027 antagonized endotoxin to a somewhat greater extent than it antagonized arachidonate (Fig. 2, Table Ib), but this difference was not statistically significant.

When L 8027 was given 30 min before prostaglandin E_1, it produced no statistically significant change in the hyperthermic response (Fig. 3, Table Ic).

Discussion. L 8027 was a very effective antipyretic with a potency in antagonizing bacterial endotoxin intermediate between that of indomethacin (6, 13) and acetaminophen (6, 14). Relative to their abilities to antagonize endotoxin, L 8027 produced about twice the reduction in arachidonate-induced hyperthermia that acetaminophen did. For comparison, Table II shows the decreases in response to both arachidonate and endotoxin produced by four antipyretics. The

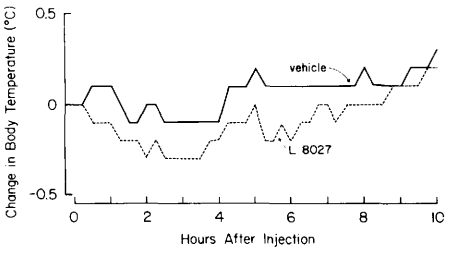

FIG. 1. Hypothermic effect of L 8027. Mean responses of six afebrile cats to iv injection of ethanol vehicle or 2 mg/kg L 8027.

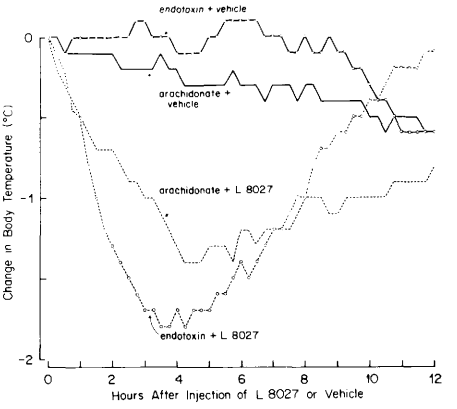

FIG. 2. Reduction of hyperthermic responses to intraventricular injections of bacterial endotoxin (0.05 μg) and sodium arachidonate (50–200 μg) by 2 mg/kg L 8027 given iv. In this study with six cats, L 8027 or ethanol was given after hyperthermia had reached maximal levels.

TABLE I. EFFECT OF L 8027 ON BODY TEMPERATURE OF AFEBRILE AND HYPERTHERMIC CATS. RESULTS ARE EXPRESSED AS MEAN ± SE IN SIX CATS.

Experiment no.	Hyperthermogenic agent	Body temperature (°C) at time of L 8027 or ethanol injection		Time period for TRI calculation (hr)	TRI[a] (Δ° × hr)		
		Control	L 8027		Control	L 8027	Δ
Ia)	none	38.6 ± 0.3	38.4 ± 0.1	0–8	0.3 ± 0.7	−1.2 ± 0.4	−1.4 ± 0.5*
Ib)	endotoxin	40.8 ± 0.3	40.3 ± 0.2	total	−0.8 ± 1.6	−13.6 ± 4.3	−12.9 ± 3.2**
	arachidonate	40.5 ± 0.4	40.2 ± 0.2		−3.7 ± 3.0	−12.2 ± 3.4	−8.5 ± 2.1**
Ic)	prostaglandin E_1	38.5 ± 0.1	38.2 ± 0.2	0–4	3.2 ± 0.7	4.0 ± 0.6	0.7 ± 0.9

[a] TRI = Thermal response index; see Materials and Methods section.

* $P < 0.05$.

** $P < 0.01$ by paired t test.

basic antipyretics were more effective antagonists of arachidonate relative to endotoxin than were the acidic antipyretics. The 5-hr TRI for L 8027 is shown to facilitate comparison with the other antipyretics which had shorter durations of action. In accordance with the evidence that prostaglandins may not be solely responsible for arachidonate-induced hyperthermia (7), this relatively greater ability of L 8027, and perhaps of tilorone, to antagonize arachidonate may reflect inhibition of both prostaglandin and thromboxane synthetases, provided that thromboxane does contribute to the hyperthermia. Alternatively the basic antipyretics may inhibit synthesis of other arachidonate derivatives.

Intraventricular injection of prostacyclin, another such derivative, can cause hyperthermia in the cat (15). Prostacyclin was between 1/100 and 1/1000 as potent as prostaglandin E_1, probably because of its relative instability. The ability to antagonize arachidonate-induced hyperthermia might be especially useful in treatment of hyperthermic responses to brain damage. Rudy *et al.* (16) have recently reported that injury to the hypothalamus of the rat results in hyperthermia which can be antagonized by large doses of indomethacin and which, therefore, may involve release of cyclic endoperoxide derivatives. The ability of L 8027 to antagonize arachidonate was not due to a direct antagonism of prostaglandin since L 8027 did not antagonize injected prostaglandin. Antagonism of endotoxin and arachidonate was not the result of a hypothermogenic action of L 8027 since L 8027 had only a slight effect on body temperature of afebrile animals. These results indicate that L 8027 is a relatively potent antipyretic which can also antagonize arachidonate-induced hyperthermia.

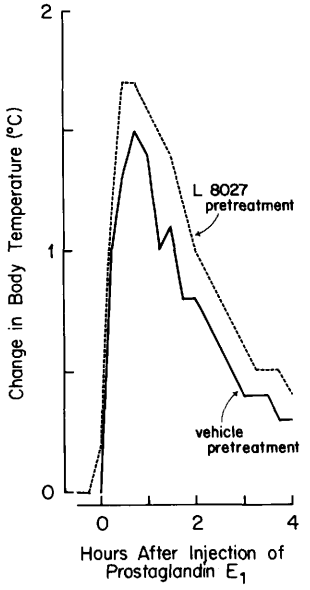

FIG. 3. Mean responses of six cats to intraventricular administration of prostaglandin E_1 (0.15–0.50 μg) preceded 30 min by ethanol vehicle or 2 mg/kg L 8027 iv.

Summary. The ability of L 8027 to antagonize hyperthermic responses to a bacterial pyrogen, sodium arachidonate and prostaglandin E_1 was studied in unanesthetized, unrestrained cats. The hyperthermogenic agents were injected into the third cerebral ventricle. L 8027, 2 mg/kg in 0.10 ml/kg 95% ethanol, was given iv. When L 8027 was administered during hyperthermic responses to *Salmonella typhosa* endotoxin and sodium arachidonate, both were significantly antagonized, the endotoxin to a somewhat greater extent. When L 8027 was given 30 min prior to prostaglandin E_1, the response to the prostaglandin was not altered significantly. L 8027 given alone lowered body temperature approximately 0.2° for 8 hr when compared to the temperature change after injection of

TABLE II. ANTAGONISM OF ARACHIDONATE AND ENDOTOXIN BY ANTIPYRETICS.

	Antipyretic	Dose (mg/kg)	Time period (hr)	Reduction from control response Δ TRI[a] (Δ° × hr) Arachidonate	Endotoxin	Ratio	Ref.
acidic	acetaminophen	40	0–5	1.8	5.6	0.32	6
acidic	indomethacin	0.04	0–5	1.0	2.6	0.38	6
	tilorone	10	0–6	3.4	2.9	1.17	10
basic	L 8027	2	0–5	3.5	6.3	0.56	
	L 8027	2	total	8.5	12.9	0.66	

[a] TRI = Thermal response index; see Materials and Methods Section.

vehicle. These results indicate that L 8027 is a relatively potent antipyretic which can also inhibit arachidonate-induced hyperthermia.

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1. Brune, K., Glatt, M., and Graf, P., Gen. Pharmacol. **7**, 27 (1976).
2. Colot, M., Van Damme, M., Dirks, M., Beersaerts, J., Deridder, G., and Charlier, R., Thérapie **28**, 775 (1973).
3. Deby, C., Descamps, M., Binon, F., and Bacq, Z. M., Biochem. Pharmacol. **24**, 1089 (1975).
4. Gryglewski, R. J., Zmuda, A., Korbut, R., Krecioch, E., and Bieron, K., Nature (London) **267**, 627 (1977).
5. Spławiński, J. A., Reichenberg, K., Vetulani, J., Marchaj, J., and Kałuża, J., Pol. J. Pharmacol. Pharm. **26**, 101 (1974).
6. Clark, W. G., and Cumby, H. R., J. Physiol. **257**, 581 (1976).
7. Laburn, H., Mitchell, D., and Rosendorff, C., J. Physiol. **267**, 559 (1977).
8. Kandasamy, S. B., Experientia **33**, 1626 (1977).
9. Hawkins, M., and Lipton, J. M., Prostaglandins **13**, 209 (1977).
10. Clark, W. G., and Robins, J. A., Brit. J. Pharmacol. **62**, 281 (1978).
11. McCarthy, L. E., and Borison, H. L., Anat. Rec. **155**, 305 (1966).
12. Clark, W. G., and Moyer, S. G., J. Pharmacol. Exp. Ther. **181**, 183 (1972).
13. Clark, W. G., and Cumby, H. R., J. Physiol. **248**, 625 (1975).
14. Clark, W. G., J. Pharmacol. Exp. Ther. **175**, 469 (1970).
15. Clark, W. G., and Lipton, J. M., Brain Res. Bull. **4** (1979), in press.
16. Rudy, T. A., Williams, J. W., and Yaksh, T. L., J. Physiol. **272**, 721 (1977).

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