Pharmacology Biochemistry & Behavior, Vol. 25, pp. 347–351, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Examination of the Subdiencephalic Rat Brain for Sites Mediating PGE_1-Induced Pyrexia

O. E. OLORUNDARE AND T. A. RUDY[1]

School of Pharmacy, University of Wisconsin, Madison, WI 53706

Received 3 January 1986

OLORUNDARE, O. E. AND T. A. RUDY. *Examination of the subdiencephalic rat brain for sites mediating PGE_1-induced pyrexia.* PHARMACOL BIOCHEM BEHAV **25**(2) 347–351, 1986.—The subdiencephalic rat brain was mapped for sites capable of mediating prostaglandin-induced pyrexia. In conscious rats, PGE_1, 200 ng in a volume of 1 μl, was injected unilaterally into 412 sites between the midmesencephalon and the caudal medulla. Injections into only 12 sites caused a reproducible, short-latency core temperature increase of at least 0.5°C. None of these was located in the paramedian brainstem, which was considered a likely site of PGE_1 action because of the presence there of thermosensitive and pyrogen-sensitive neurons. Rather, the reactive loci were found in the hippocampus (5 sites) and in the vicinity of the cochlear nuclei (7 sites). Injections into only 2 sites in the latter region failed to produce pyrexia. In the hippocampus, however, injections at 31 sites in the same frontal planes as the reactive loci produced no effect. The possibility that the active hippocampal sites were associated with a distribution of injectate to PGE_1-sensitive neurons located within hippocampal cleavage planes rather than in a circumscribed region is discussed.

Rat PGE_1 Fever/pyrexia Brainstem Hippocampus Cochlear nuclei

THERE is an abundance of evidence that pyrogen-induced fevers are mediated ultimately by an action in the brain of cyclooxygenase products, in particular, prostaglandins of the E series [11]. It also seems likely that prostaglandins mediate fevers associated with acute hypothalamic trauma and with intraventricular hemorrhage [1, 35, 36]. It is generally assumed that the pyrexic effect of prostaglandins is due to an action upon neurons within the anterior hypothalamic/preoptic region (AH/PO). This assumption is based on the well-known importance of this region in the control of body temperature and upon "mapping" studies in which E series prostaglandins injected into the AH/PO produced hyperthermia, whereas injections into nearby loci did not (see [5] for references).

There is reason to believe, however, that prostaglandins may produce pyrexia through an action at a site or sites outside the AH/PO. Monkeys in which the AH/PO had been completely destroyed bilaterally nevertheless experienced strong pyrexia after IV injection of endogenous pyrogen or intracerebroventricular injection of bacterial endotoxin or PGE_1 [23]. A retention of at least some responsiveness to circulating pyrogen after large, histologically verified bilateral lesions of the AH/PO or after transection of the brainstem at the midbrain level has also been observed in goat [2], rabbit [6], cat [4], and man [22]. In rabbits and rats in which the AH/PO was intact, injection of leukocytic pyrogen and bacterial endotoxin, respectively, into the lower brainstem produced a body temperature increase [34,39]. Although prostaglandins were not injected in these animals, the known ability of pyrogens to release prostaglandins from brain tissue [10] suggests that the pyrogen effects might have been mediated through the local release of prostaglandins. Finally, populations of thermosensitive neurons have been found in the lower brainstem of several species ([8, 9, 19, 21, 38]; see [32] for additional references). The pyrexic effect of prostaglandins injected into the AH/PO is probably mediated by an action on thermosensitive units [12, 13, 18]. There is thus a possibility that lower brainstem thermosensitive neurons are similarly responsive.

In a previous investigation, we injected a fixed dose of PGE_1 into many sites within the rat brain and found that only injections in or very near the AH/PO produced a temperature increase [44]. However, relatively few sites below the midmesencephalic level were examined. Although others have investigated in various species the pyrexic effect of E series prostaglandins injected into the subdiencephalic brain [14, 24, 40, 41], these studies were limited in scope. In view of the possibility that a site capable of mediating prostaglandin-induced hyperthermia exists in this portion of the brain, an extensive exploration of subdiencephalic tissue for PGE_1-sensitive sites was carried out in the present study.

METHOD

Forty albino rats (King Animal Labs, Oregon, WI) weighing 280–320 g at the time of surgery were used. Under pen-

[1]Requests for reprints should be addressed to Dr. Thomas A. Rudy.

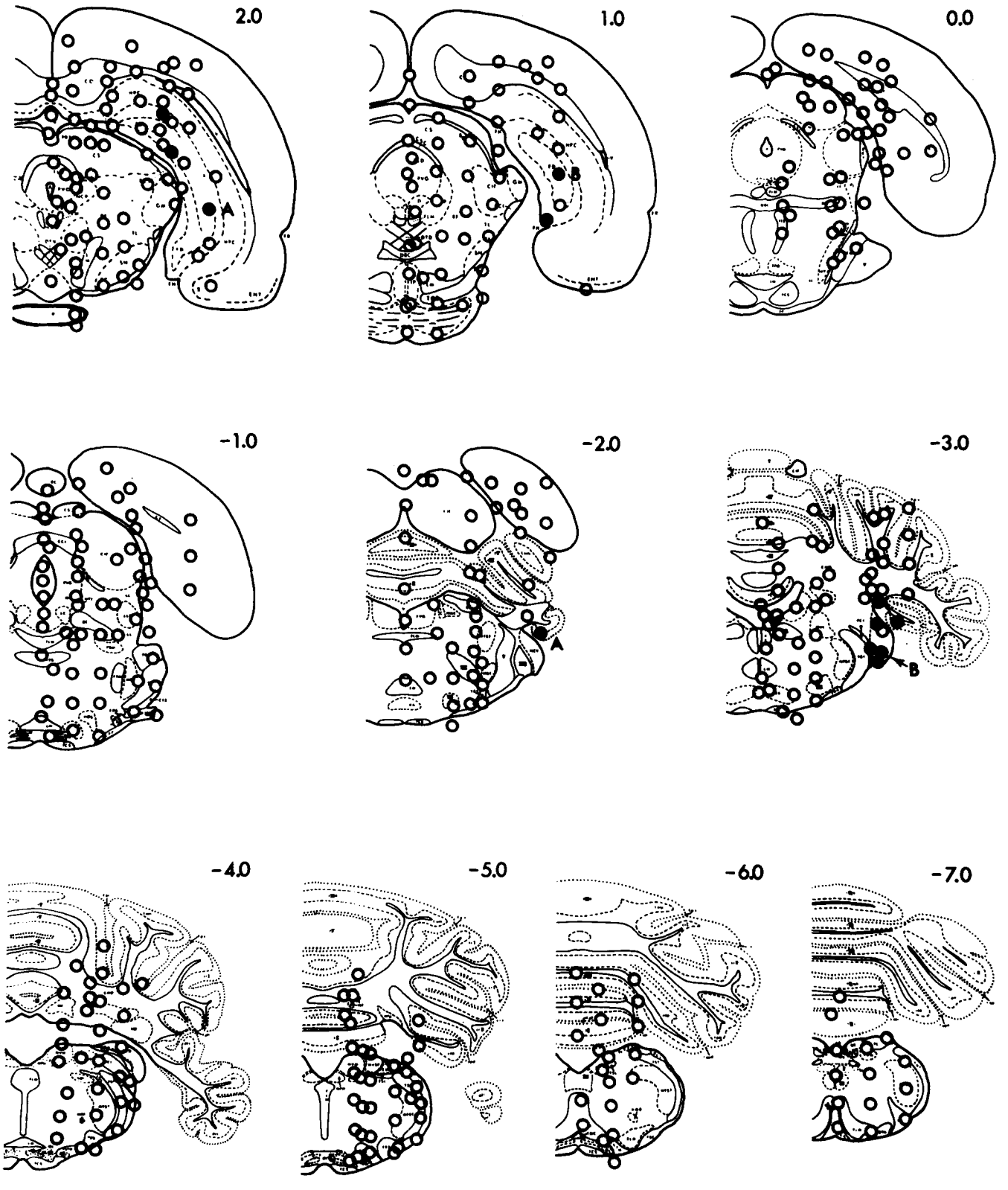

FIG. 1. Frontal sections of the rat brain illustrating the approximate positions of the PGE_1 injection sites. Open circles: unresponsive sites. Filled circles: responsive sites (see text for further explanation). A and B in planes 2.0 and 1.0 denote the sites whose responses to PGE_1 are shown in Fig. 2. A and B in planes −2.0 and −3.0 denote the sites whose responses to PGE_1 are shown in Fig. 3.

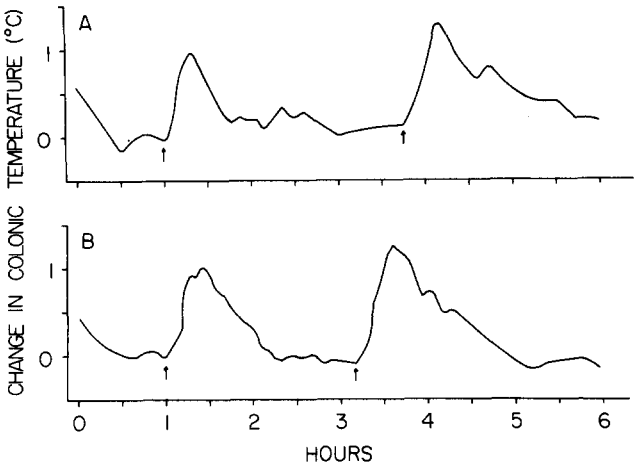

FIG. 2. Core temperature increases produced by injection of 200 ng PGE_1 into 2 responsive sites in the hippocampus. Arrows indicate times of injection. The positions of the 2 sites are shown in Fig. 1, AP planes 2.0 and 1.0.

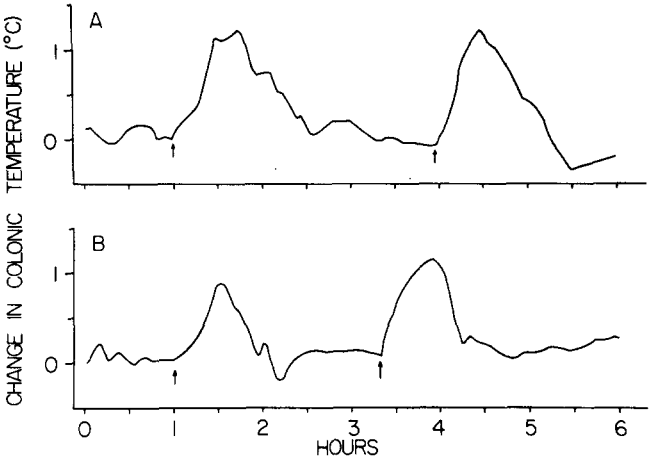

FIG. 3. Core temperature increases produced by injection of 200 ng PGE_1 into 2 responsive sites in the cochlear nucleus region. Arrows indicate times of injection. The positions of the 2 sites are shown in Fig. 1, AP planes −2.0 and −3.0.

tobarbital anesthesia (35 mg/kg IP) and using the stereotaxic atlas of Pellegrino, Pellegrino and Cushman [29], 1 to 3 stainless steel injection guides (24 gauge, thinwall) were implanted in the brain of each animal and secured to the skull with stainless steel screws and dental acrylic. The tips of most of the guides were placed high in the brain so that intracerebral injections could be made at several successive 1 mm depths of penetration below the guide tip. In rats with more than one guide, the cannulae were positioned to minimize the possibility of producing bilateral damage to any given structure. Following implantation, each guide was occluded with a stainless steel stylet. At least 5 days were permitted for recovery from surgery.

During experimental sessions, each rat was restrained in a loosely fitting, hemicylindrical wire mesh cage at an ambient temperature of 23.5±1.5°C. A thermistor probe was inserted into the colon 6 cm beyond the anus and the lead taped to the rat's tail. Continuous records of colonic temperature (T_c) were obtained using a bridge circuit and a potentiometric recorder. At the beginning of the session, the stylet was removed from the guide tube to be used and replaced with a 29 gauge injector cannula of appropriate length. The injector was connected by polyethylene tubing (PE-10) to a gas-tight Hamilton microsyringe. The injector and tubing had been filled previously with the solution to be injected and the syringe, with 70% alcohol. A small air bubble separated the two liquids. After T_c had stabilized (1–2 hr), 1 μl of fluid was injected over a 2 min period. In most sessions, the injector was left in position and 1 or 2 subsequent injections of the same substance (PGE_1 or 0.9% saline) were made at the same site. A minimum of 1 hr separated these injections. In some sessions, the original injector was then removed and replaced with another of identical length loaded with a different substance. In these instances, T_c was permitted to restabilize before the second substance (0.9% saline or PGE_1) was administered.

A minimum of 2 injections of a solution containing 200 ng of PGE_1 sodium was made into each available site in each rat. PGE_1 (Upjohn Diagnostics) was reacted with a stoichiometric amount of Na_2CO_3 to form a stock solution (1 mg/ml in 0.9% saline) which was stored at −70°C and diluted with 0.9% saline as needed. At sites where PGE_1 injections produced a temperature rise, 0.9% saline was also injected at least once. Within each guide track, experiments at each depth were completed before moving on to the next lower depth. Following the completion of all experiments in a rat, the animal was anesthetized and the brain fixed by intracardiac injection of 10% formalin. Transverse frozen sections of the brian were prepared, stained with cresyl violet and examined for the position of the injection sites.

RESULTS

In Fig. 1 are shown the approximate positions of the 412 sites tested with PGE_1. Filled circles represent sites where each of 2 consecutive injections of PGE_1 produced a temperature increase of at least 0.5°C which began within 5 min after injection and where an injection of 0.9% saline produced a temperature increase of less than 0.2°C. Open circles represent sites where PGE_1 failed to produce a criterion level temperature rise. Only 12 sites were responsive to PGE_1. Five of these were located in the hippocampus and 7 in or near the cochlear nuclei. Examples of responses elicited by hippocampal and cochlear nucleus injections are shown in Fig. 2 and 3.

DISCUSSION

In this study, a large standard dose of PGE_1 (200 ng) was used so that even weakly responsive loci might be detected. This is at least 4 times [44] and perhaps as much as 100 times [41] the dose of PGE_1 required to produce hyperthermia when injected into the AH/PO region of the rat. Nevertheless, injections at 97% of the sites failed to produce a body temperature increase. A region which is noteworthy because of its lack of responsiveness to PGE_1 is the brainstem core between the midpoint of the midbrain and the caudal medulla. Paramedian structures within this region in several species exhibit thermosensitivity ([8, 9, 19, 21, 38]; see [32] for additional references) and/or sensitivity to intravenously or intracerebrally injected endogenous pyrogen or bacterial

endotoxin [25, 34, 37, 39] and are thus likely sites of prostaglandin action. Although there are a few gaps in coverage (e.g., midline structures at AP −4.0 and −5.0), we made injections of PGE_1 into most aspects of the core of the lower brainstem. Our negative findings are in accord with those of others who have carried out limited explorations with E series prostaglandins of the paramedian midbrain [40,41] and medulla [14,24]. The results support the contention that some CNS populations of thermosensitive units either do not respond to E series prostaglandins or respond in a way which differs from thermosensitive units in the AH/PO [24,44]. Our data are also compatible with the suggestion that, at some CNS sites of action, pyrogens can cause fever through a mechanism not involving prostaglandins [6, 7, 30, 31, 44]. One must consider an alternative interpretation, however, and that is that the PGE_1 dose we used, albeit large, was distributed inadequately. It is possible that prostaglandin sensitive units in the brain stem are diffusely distributed and that our discrete injections of PGE_1 may have failed to activate a volume of tissue sufficient to elicit a temperature increase. It thus remains a possibility that an effect of prostaglandins on the lower brainstem core contributes to fever production in situations in which a large volume of tissue is exposed to these substances. Events which could produce such widespread exposure include intracranial infection, certain types of cerebral injury and, perhaps, the presence of high circulating titers of endogenous pyrogen.

The few sites wherein PGE_1 injections did produce pyrexia were located in or near the cochlear nuclei and in the hippocampus. Although for all responsive sites, the latency was short and the temperature rose rapidly, the maximum core temperature increase was not large (see Figs. 2 and 3). Since the dose of PGE_1 administered was considerably greater than that required to produce hyperthermia when injected into the AH/PO region [41,44], it seems safe to say that these extra-AH/PO sites are less sensitive to PGE_1 than the AH/PO region. Cochlear nucleus injections produced pyrexia with good reliability; 7 of 9 sites in that region were responsive. However, the cochlear nuclei have no known involvement in fever production or in normal thermoregulation. Futhermore, the cochlear nuclei protrude into the subarachnoid space, increasing the likelihood that injectate could enter the cerebrospinal fluid and be carried forward to the AH/PO. This possibility and other matters relating to the putative cochlear nucleus site of action are considered in detail in a companion paper [27]. In brief, the results of that paper confirm that PGE_1 injections into the cochlear nucleus region reliably produce pyrexia and suggest that transport of PGE_1 to the AH/PO is not responsible for the temperature rise.

PGE_1 injections into the hippocampus produced pyrexia with less reliability than injections near the cochlear nuclei. Only 5 of the 36 sites which were in or very near the hippocampus were reactive to PGE_1, and these 5 sites were not clustered in a circumscribed area. The reason why only a few sites were responsive is not obvious, but one possible explanation relates to the convoluted structure of the hippocampus. In some instances, the injectate may have infiltrated along hippocampal cleavage planes, whereas in most injections the solution was distributed in the more typical spherical form. If this is the case, it would suggest that PGE_1 sensitive neurons in the hippocampus are distributed in a planar fashion rather than being congregated in discrete regions. Another possibility is that planar flow led the injectate toward the lateral ventricle or the subarachnoid space where it could then be transported to the AH/PO. This seems unlikely, however, because numerous injections directly into or quite near the lateral ventricle or the subarachnoid space in the same frontal planes as the responsive hippocampal sites failed to produce a temperature increase.

Although the reactivity of the hippocampus to PGE_1 requires further documentation, there is good reason to believe that the hippocampus participates in the control of body temperature and that modulation of its activity can have important thermoregulatory effects. There are abundant neuronal connections passing in both directions between the hippocampus and the hypothalamus [26,33]. Hippocampal EEG activity is influenced by changes in skin [17], body core [43] and hypothalamic [16] temperatures. Electrical stimulation of the hippocampus preferentially alters the firing rates of preoptic and septal temperature sensitive neurons in comparison to temperature insensitive units [3, 15, 28], produces changes in body temperature [28] and elicits "wet dog shakes" [20], a phenomenon believed by some to be a form of shivering [42].

ACKNOWLEDGEMENTS

This investigation was supported by Grant NS 19112 from the National Institutes of Health, United States Public Health Service and by contract N00014-75-0939, United States Office of Naval Research.

REFERENCES

1. Ackerman, D. and T. A. Rudy. Thermoregulatory characteristics of neurogenic hyperthermia in the rat. *J Physiol* **307**: 59–70, 1980.
2. Andersson, B., C. C. Gale, B. Hökfelt and B. Larsson. Acute and chronic effects of preoptic lesions. *Acta Physiol Scand* **65**: 45–60, 1965.
3. Boulant, J. A. and H. N. Demieville. Responses of thermosensitive preoptic and septal neurons to hippocampal and brain stem stimulation. *J Neurophysiol* **40**: 1356–1368, 1977.
4. Chambers, W. W., H. Koenig, R. Koenig and W. F. Windle. Site of action in the central nervous system of a bacterial pyrogen. *Am J Physiol* **1959**: 209–216, 1949.
5. Clark, W. G. and Y. L. Clark. Changes in body temperature after administration of acetylcholine, histamine, morphine, prostaglandins and related agents. *Neurosci Biobehav Rev* **4**: 175–240, 1980.
6. Cooper, K. E., W. L. Veale and Q. J. Pittman. Pathogenesis of fever. In: *Brain Dysfunction in Infantile Febrile Convulsions*, edited by M. A. B. Brazier and F. Coceani. New York: Raven Press, 1976, pp. 107–115.
7. Cranston, W. I., G. W. Duff, R. F. Hellon, D. Mitchell and Y. Townsend. Evidence that brain prostaglandin synthesis is not essential in fever. *J Physiol* **259**: 239–249, 1976.
8. Cronin, M. J. and M. A. Baker. Thermosensitive midbrain neurons in the cat. *Brain Res* **128**: 461–472, 1977.
9. Cronin, M. J. and M. A. Baker. Physiological responses to midbrain thermal stimulation in the cat. *Brain Res* **128**: 542–546, 1977.
10. Dinarello, C. A. and H. A. Bernheim. The ability of human leukocyte pyrogen to stimulate brain prostaglandin synthesis in vitro. *J Neurochem* **37**: 702–708, 1981.

11. Dinarello, C. A. and S. M. Wolff. Molecular basis of fever in humans. *Am J Med* **72:** 799–819, 1982.
12. Ford, D. M. A selective action of prostaglandin E_1 on hypothalamic neurons in the cat responding to brain cooling. *J Physiol* **242:** 142P–143P, 1974.
13. Gordon, C. J. and J. E. Heath. The effect of prostaglandin E_2 on the firing rate of thermal sensitive and insensitive neurons in the preoptic/anterior hypothalamus of unanesthetized rabbits. *Fed Proc* **38:** 1295, 1979.
14. Hawkins, M. and J. M. Lipton. Analogs of endoperoxide precursors of prostaglandins: failure to affect body temperature when injected into primary and secondary central temperature controls. *Prostaglandins* **13:** 209–218, 1977.
15. Hori, T., T. Osaka, T. Kiyohara, M. Shibata and T. Nakashima. Hippocampal input to preoptic thermosensitive neurons in the rat. *Neurosci Lett* **32:** 155–158, 1982.
16. Horowitz, J. M. and M. A. Saleh. Response of a hippocampal neural network to changes in hypothalamic temperature in the rabbit. Satellite Symp. Thermal Physiol, Pécs, Hungary, 1980, p. 25. (Abstract).
17. Horowitz, J. M., M. A. Saleh and R. D. Karem. Correlation of hippocampal theta rhythm with changes in cutaneous temperature. *Am J Physiol* **227:** 635–642, 1974.
18. Jell, R. M. and P. Sweatman. Prostaglandin-sensitive neurons in cat hypothalamus: relation to thermoregulation and to biogenic amines. *Can J Physiol Pharmacol* **55:** 560–567, 1977.
19. Kobayashi, S. and N. Murakami. Thermosensitive neurons in slice preparations of rat medulla oblongata. *Brain Res Bull* **8:** 721–726, 1982.
20. Le Gal La Salle, G. and E. A. Cavalheiro. Stimulation of septal and amygdaloid nuclei: EEG and behavioral responses during early development of kindling with special reference to wet dog shakes. *Exp Neurol* **74:** 717–727, 1981.
21. Lipton, J. M. and J. I. Kennedy. Central thermosensitivity during fever produced by intra-PO/AH and intravenous injections of pyrogen. *Brain Res Bull* **4:** 23–34, 1979.
22. Lipton, J. M., J. Kirkpatrick and R. N. Rosenberg. Hypothermia and persisting capacity to develop fever. *Arch Neurol* **34:** 498–504, 1977.
23. Lipton, J. M. and G. P. Trzcinka. Persistence of febrile response to pyrogens after PO/AH lesions in squirrel monkeys. *Am J Physiol* **231:** 1638–1648, 1976.
24. Lipton, J. M, J. P. Welch and W. G. Clark. Changes in body temperature produced by injecting prostaglandin E_1, EGTA and bacterial endotoxins into the PO/AH region and medulla oblongata of the rat. *Experientia* **29:** 806–808, 1973.
25. Nakayama, T. and T. Hori. Effects of anesthetic and pyrogen on thermally sensitive neurons in the brainstem. *J Appl Physiol* **34:** 351–355, 1973.
26. Nauta, W. J. H. Hippocampal projections and related neural pathways to the midbrain in the cat. *Brain* **81:** 319–340, 1958.
27. Olorundare, O. E. and T. A. Rudy. Studies on the pyrexic effect of PGE_1 injected into the region of the cochlear nuclei. *Pharmacol Biochem Behav* **25:** 353–358, 1986.
28. Osaka, T., T. Hori, T. Kiyohara, M. Shibata and T. Nakashima. Changes in body temperature and thermosensitivity of preoptic and septal neurons during hippocampal stimulation. *Brain Res Bull* **13:** 93–98, 1984.
29. Pellegrino, L. J., A. S. Pellegrino and A. J Cushman. *A Stereotaxic Atlas of the Rat Brain*, 2nd edition. New York: Plenum Press, 1979.
30. Pittman, Q. J., W. L. Veale and K. E. Cooper. Absence of fever following intrahypothalamic injections of prostaglandins in sheep. *Neuropharmacology* **16:** 743–749, 1977.
31. Pittman, Q. J., W. L. Veale and K. E. Cooper. Effect of prostaglandin, pyrogen and noradrenaline, injected into the hypothalamus, on thermoregulation in newborn lambs. *Brain Res* **128:** 473–483, 1977.
32. Reaves, T. R. and J. N Hayward. Hypothalamic and extrahypothalamic thermoregulatory centers. In: *Body Temperature*, edited by P. Lomax and E. Schonbaum. New York: Marcel Dekker, 1979, pp. 39–70.
33. Riley, J. N. and R. Y. Moore. Diencephalic and brainstem afferents to the hippocampal formation of the rat. *Brain Res Bull* **6:** 437–444, 1981.
34. Rosendorff, C. and J. J. Mooney. Central nervous system sites of action of a purified leucocyte pyrogen. *Am J Physiol* **220:** 597–603, 1971.
35. Rudy, T. A. and H. M. Gollman. The role of cyclooxygenase products in fever elicited by intraventricular injection of serum derived from aged blood. *Fed Proc* **43:** 910, 1984.
36. Rudy, T. A., J. Williams and T. L. Yaksh. Antagonism by indomethacin of neurogenic hyperthermia produced by unilateral puncture of the anterior hypothalmic/preoptic region. *J Physiol* **272:** 721–736, 1977.
37. Sakata, Y. Effects of pyrogen on the medullary temperature-responsive neurone of rabbits. *Jn J Physiol* **29:** 585–596, 1979.
38. Schmieg, G., J. B. Mercer and C. Jessen. Thermosensitivity of the extra-hypothalamic brain stem in conscious goats. *Brain Res* **188:** 383–397, 1980.
39. Splawiński, J. A., Z. Górka, E. Zacny and J. Kaluża. Fever produced in the rat by intracerebral E. coli endotoxin. *Pflugers Arch* **368:** 117–123, 1977.
40. Stitt, J. T. Prostaglandin E_1 fever induced in rabbits. *J Physiol* **232:** 163–179, 1973.
41. Veale, W. L. and I. Q. Whishaw. Body temperature responses at different ambient temperatures following injections of prostaglandin E_1 and noradrenaline into the brain. *Pharmacol Biochem Behav* **4:** 143–150, 1976.
42. Wei, E. T. Pharmacological aspects of shaking behavior produced by TRH, Ag-3-5, and morphine withdrawal. *Fed Proc* **40:** 1491–1496, 1981.
43. Whishaw, I. Q. and C. H. Vanderwolf. Hippocampal EEG and behavior: Effects of variation in body temperature and relation of EEG to vibrissae movement, swimming and shivering. *Physiol Behav* **6:** 391–397, 1971.
44. Williams, J. W., T. A. Rudy, T. L. Yaksh and C. T. Viswanathan. An extensive exploration of the rat brain for sites mediating prostaglandin-induced hyperthermia. *Brain Res* **120:** 251–262, 1977.