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Studies on the Pyrexic Effect of PGE_1 Injected Into the Region of the Cochlear Nuclei

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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(2) 353–358, 1986.—In 30 of 33 unanesthetized rats, unilateral injection of PGE_1 (100 ng in 1 μl) into or near the cochlear nuclei (CN) produced a body temperature increase of at least 0.5°C. Usually, the rise started within the first minute after injection. Bilateral destruction of the highly PGE_1-sensitive anterior hypothalamic/preoptic region (AH/PO) eliminated the response. In approximately two-thirds of rats in which concentrated dye or ^{3}H-PGE_1 was injected into CN, there was evidence that a small portion of the injectate had rapid access to the AH/PO. In the remaining rats, the tracer material did not reach AH/PO tissue. In the rats involved in the tracer studies, there was no correlation between the magnitude of the response to PGE_1 injected into CN and the extent of transport of tracer from CN to AH/PO. The results suggest that PGE_1 injected into CN produced pyrexia through an action on tissue at or near the injection site and that neuronal pathways in AH/PO are necessary for the expression of the response.

PGE_1 Fever/pyrexia Cochlear nuclei Anterior hypothalalmus Preoptic region Rat

IN a previous publication from this laboratory [8], in which the subdiencephalic portion of the rat brain was explored for sites wherein an injection of PGE_1 could elicit hyperthermia, it was noted that unilateral injection of 200 ng of PGE_1 into or near the cochlear nuclei caused a core temperature increase. Similar injections into numerous other loci (with the exception of the hippocampus) produced no effect. The goals of the present study were to document the responsiveness of the cochlear nucleus region (CN) more extensively and to examine the possibility that the response following cochlear nucleus injections is mediated by transport of the injected PGE_1 to the anterior hypothalamic/preoptic region (AH/PO), in particular via the subarachnoid cerebrospinal fluid (CSF), into which the acoustic tubercle protrudes. The AH/PO region is known to be highly sensitive to pyrogenic prostaglandins such as PGE_1 (see [3] for references).

METHOD

Male albino rats (King Animal Labs, Oregon, WI) weighing 280–320 g at the time of surgery were used. A guide cannula through which injections were made into the brain substance, the cerebral ventricles or the subarachnoid space was implanted in each rat. Rats were anesthetized with pentobarbital (35 mg/kg IP) and placed in a Kopf stereotaxic instrument. Using the stereotaxic atlas of Pellegrino, Pellegrino and Cushman [10] (and the atlas of Paxinos and Watson [9] for interpeduncular cistern implants), the guide cannula (stainless steel, 24 gauge thin wall) was inserted and secured to the skull with stainless steel screws and dental acrylic. The following coordinates were employed for the various sites tested: anterior hypothalamic/preoptic region (AP 7.8, L 1.0, H 0.0), dorsal third ventricle/cerebral aqueduct junction (AP 3.0, L 0.0, H 0.5), interpeduncular cistern (AP 3.3, L 0.1, H 2.6), cochlear nucleus region (CN) (AP −3.0 to −2.2, L 3.8 to 4.1, H −2.5 to −3.5). The implanted guides were occluded with stainless steel stylets. Five days were allowed for recovery before experiments were begun.

In some rats, the AH/PO was destroyed bilaterally at the same time that a guide cannula was implanted into the dorsal third ventricle or interpeduncular cistern. Two holes overlying the intended targets were drilled in the skull. The lesioning electrode (Kopf model K1388Z) was inserted into each hole in turn to the coordinates AP 8.4, L 0.5, H −2.5 [10]. Using a Kopf model RFG-4 radiofrequency lesion maker, the electrode tip temperature was increased over 15 sec to 72.5°C and then maintained at that level for an additional 60 sec. To reduce postoperative mortality, the rats were kept at an ambient temperature of 15°C during the first 24 postlesioning hours [6].

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 6 cm into the colon and held in place by taping the lead to the rat's tail. Colonic temperature was recorded continuously using a bridge circuit and a potentiometric recorder. At the beginning of the session, the guide cannula stylet was removed and replaced with a 29 gauge stainless steel injector

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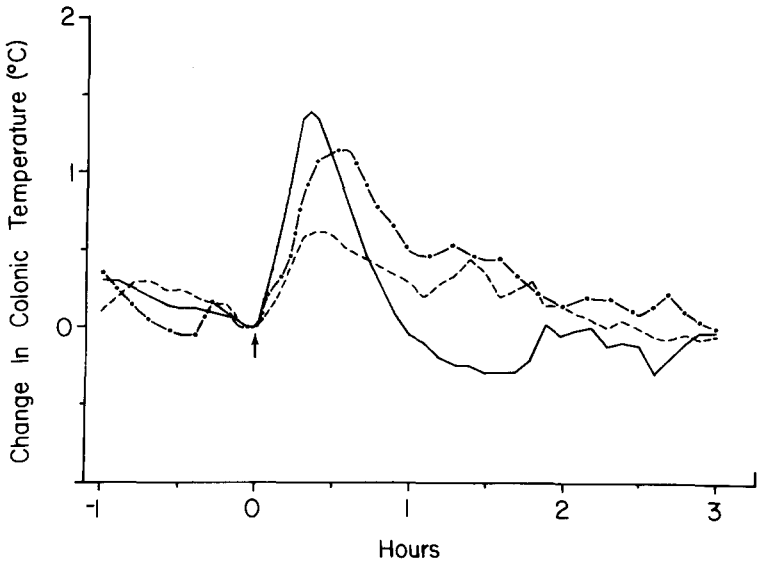

FIG. 1. The effect on colonic temperature of an injection of 100 ng PGE_1 into the cochlear nucleus region in 3 individual rats. Arrow denotes the end of the 2-min PGE_1 injection period.

cannula which protruded 2 mm beyond the guide cannula tip. In the dorsal third ventricle/cerebral aqueduct junction injections, insertion of the injector occluded the aqueduct, ensuring that the injectate was confined within the third ventricle. Polyethylene tubing (PE-10) connected the injector cannula to a gas-tight Hamilton microsyringe. Prior to lowering the injector cannula into its guide, the syringe was filled with 70% alcohol and the cannula and tubing with the solution to be injected. A small air bubble separated the two liquids. After colonic temperature had stabilized (1–2 hr), 1 μl of the fluid was injected over a period of 2 min. The injector cannula was left in place for the remainder of the session. For all experiments except the ^{3}H-PGE_1 diffusion studies (vide infra), the injectate consisted of PGE_1 sodium (100 ng/μl) or 0.9% saline. PGE_1 was obtained from Upjohn Diagnostics, Kalamazoo, MI. A stock solution of the sodium salt (1 mg/ml) in 0.9% saline was prepared by stoichiometric reaction with Na_2CO_3 and stored frozen at −70°C. Dilutions in 0.9% saline for injection were prepared as needed.

Changes in colonic temperature evoked by treatments were quantified on the basis of latency to onset of response, maximum increase in temperature (ΔT_c) and fever index (FI_{180}). Latency was defined as the time in minutes from the end of the 2-min injection to the onset of a maintained colonic temperature increase. ΔT_c (in °C) represents the maximum change in colonic temperature occurring within 60 min after injection. FI_{180} represents the area (in °C-hr) enclosed between the fever curve and the extrapolated baseline temperature for the first 180 min following injection. Only areas above the baseline were included in the computation. Data were analyzed for statistical significance using a one-way analysis of variance (ANOVA) and Fisher's Least Significant Difference test.

TABLE 1

CHARACTERISTICS OF TEMPERATURE CHANGES PRODUCED BY INJECTION OF 100 ng PGE_1 INTO THE COCHLEAR NUCLEUS REGION (CN), THIRD CEREBRAL VENTRICLE (3V) AND INTERPEDUNCULAR CISTERN (IPC)

Site	N	Latency (min)	ΔT_c (°C)	FI_{180} (°C-hr)
CN	19	0.53 (0.25)	0.81 (0.05)	0.81 (0.09)
3V	14	0.57 (0.39)	1.10 (0.09)*	1.14 (0.17)
IPC	16	0.94 (0.37)	1.28 (0.10)*	1.24 (0.15)*

Table entries consist of mean and standard error. N=number of rats tested. For definitions of latency, ΔT_c and FI_{180}, see text. Asterisks denote values which differ significantly ($p<0.05$) from CN values for the same parameter. For all sites, the mean response to saline injections was less than 0.16°C (ΔT_c) and 0.08°C-hr (FI_{180}).

After completing the experiments in each animal, the rat was anesthetized with pentobarbital and injected at the appropriate intracranial site with 1 μl of an aqueous solution of bromophenol blue dye (5%). The brain was fixed *in situ* by intracardiac injection of 50% formalin. In those animals not having AH/PO lesions and with a guide cannula implanted at the dorsal third ventricle/aqueduct junction or the interpeduncular cistern the brain was removed immediately and cut midsagittally. The guide cannula position and the dye distribution within and on the external surface of the brain

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FIG. 2. Frontal sections of the rat brain illustrating the approximate positions of CN injection sites. Also indicated is the intensity of the hyperthermia evoked by 100 ng PGE_1 injected into each site. Intensity is quantified on the basis of the maximum increase in colonic temperature, ΔT_c, observed within the first 60 min after injection. Filled symbols: $\Delta T_c>1.0$°C; half filled symbols: $\Delta T_c=0.55$–1.0°C; open symbol: $\Delta T_c=0.45$°C. Circles: latency to onset of hyperthermia=0–3 min; squares: latency=4–10 min.

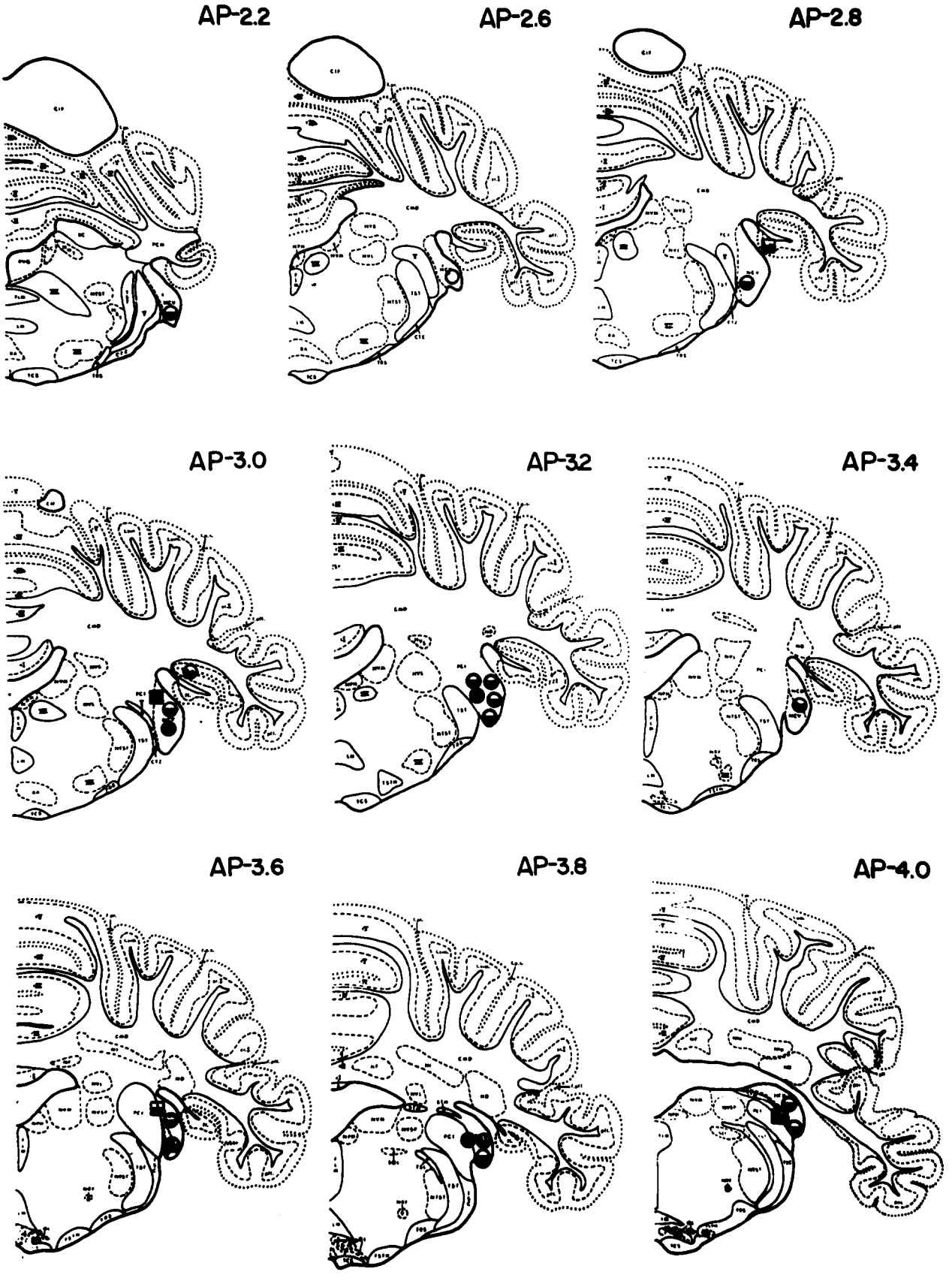
AP-2.2
AP-2.6
AP-2.8
AP-3.0
AP-3.2
AP-3.4
AP-3.6
AP-3.8
AP-4.0

TABLE 2

RESULTS OF THE ^{3}H-PGE$_1$ DIFFUSION STUDY

Rat*	% of injected dpm†	PGE$_1$ (ng)‡	ΔT_c (°C)§	FI_{180} (°C-hr)¶
1	0.000	0.000	0.80	1.11
2	0.000	0.000	1.10	1.21
3	0.000	0.000	0.80	0.38
4	0.071	0.071	0.90	0.86
5	0.243	0.243	0.20	0.11
6	0.388	0.388	0.90	0.90
7	0.487	0.487	1.05	0.83
8	0.589	0.589	0.81	0.58
9	0.608	0.608	0.40	0.52
10	1.390	1.390	0.65	0.65

*Rat identification number. Rats are listed on the basis of increasing AH/PO tritium content, not on the basis of the order in which they were injected during the experiment.

†Total percentage of injected PGE$_1$-derived tritium found in the two halves of the AH/PO tissue block.

‡Based on the percent of the injected PGE$_1$-derived tritium which reached the AH/PO in each rat, the amount of PGE$_1$ which could have reached the AH/PO following a 100 ng injection into the CN was computed.

§Maximum increase in colonic temperature produced by 100 ng PGE$_1$ injected into the CN one week before the ^{3}H-PGE$_1$ injection.

¶Fever Index produced by 100 ng PGE$_1$ injected into the CN one week before the ^{3}H-PGE$_1$ injection.

was recorded. In rats in which a guide cannula had been placed above the CN and/or which had received an AH/PO lesion, the dye distribution on the external brain surface was recorded but the brain was not cut midsagittally. Rather, frontal frozen sections were prepared, stained with cresyl violet and examined for the position of the injection site and, if appropriate, for the position and extent of the AH/PO lesion. Examinations of brains for dye distribution were usually initiated within 10 min after injection.

^{3}H-PGE$_1$ Diffusion Studies

Ten male albino rats were used. Each rat was implanted with a single guide cannula above the CN (AP −3.0, L 3.8, H −3.5). After a 5 day recovery period, rats were tested for responsiveness to PGE$_1$ as earlier described. Eight of the rats experienced a temperature increase of 0.65–1.10°C. In each responsive rat, core temperature was rising rapidly within 2 min after completion of the injection. One week later, the rats were anesthetized with pentobarbital and injected at the CN with 1 μl of a solution containing 0.1 μCi ^{3}H-PGE$_1$ (5,6-^{3}H(N)-prostaglandin E$_1$, New England Nuclear, Boston, MA) and sufficient unlabeled PGE$_1$ to produce a total concentration of 100 ng/μl. Two minutes after the injection, each animal was decaptitated, and the brain was removed rapidly and chilled for 10 min at −70°C. Blocks of tissue containing the AH/PO region were then dissected out. Cuts were made 1.5 mm rostral and caudal to the midpoint of the optic chiasm, 3 mm lateral to the midline and 3 mm above the base of the brain. Each block was separated into halves by a cut along the midline plane. Tissue samples were solubilized according to the method of Dent and Johnson [4], diluted to 15 ml with Aquasol (New England Nuclear) and assessed for tritium counts by liquid scintillation spectrometry (Packard Tricarb model 460 CD spectrometer). Quench correction was by the automatic external standard ratio method. Counting efficiency averaged 35.5%. Based on the percent of the injected ^{3}H-PGE$_1$ which was present in the AH/PO tissue blocks in each rat, the amount of unlabeled PGE$_1$ which would have reached the AH/PO following a 100 ng injection into the CN was computed. Frozen frontal sections of the posterior part of the brain of each rat were prepared and examined for the position of the injection site.

TABLE 3

CHARACTERISTICS OF TEMPERATURE CHANGES PRODUCED BY INJECTION OF 100 ng PGE$_1$ INTO THE COCHLEAR NUCLEUS REGION (CN), THIRD CEREBRAL VENTRICLE (3V) AND INTERPEDUNCULAR CISTERN (IPC) IN RATS WITH AH/PO LESIONS

Site	N	ΔT_c (°C)	FI_{180} (°C-hr)
CN	9	0.05 (0.03)	0.05 (0.02)
3V	6	0.05 (0.04)	0.02 (0.02)
IPC	8	0.07 (0.05)	0.02 (0.01)

Table entries consist of mean and standard error. N=number of rats tested. For definitions of ΔT_c and FI_{180}, see text.

RESULTS

Hyperthermia Elicited by PGE$_1$ Injected Into the Cochler Nucleus Region, Third Cerebral Ventricle and Interpeduncular Cistern

Table 1 summarizes and Fig. 1 shows representative examples of the effect on core temperature produced by injection of 100 ng of PGE$_1$ into CN in 19 rats. The characteristic response was a monophasic hyperthermia which began almost immediately after the injection was completed. The temperature increase was not accompanied by struggling, restlessness or other signs of behavioral activation. Also shown in Table 1 are the temperature changes produced by injection of the same dose of PGE$_1$ into the third ventricle and interpeduncular cistern, sites which were selected because they provide ready access of the injectate to the AH/PO. The hyperthermias produced were 1.4–1.6 times as large as those elicited by CN injections. However, there was no significant difference among the latencies to onset of hyperthermia associated with injections into the three areas.

Dye diffusion studies in the rats which received ventricular and cisternal injections revealed extensive, heavy straining of the AH/PO with no detectable staining of the CN region. Dye distribution studies in the rats which received CN injections showed three different patterns of distribution. In 7 rats (Group 1), there was no evidence of rostrad transport, the dye being confined to the CN. In another 7 rats (Group 2), the CN was intensely stained, but a faint staining of the major blood vessels on the base of the brain was noted. Among the vessels stained were those that comprise the circle of Willis, which surrounds the ventral aspect of the AH/PO. In these rats, there was no detectable staining of brain parenchyma in other than the CN. In the other 5 rats (Group 3), in addition to intense staining of the CN, there was weak, patchy staining of the surface of the infundibulum and the tissue adjacent to the optic chiasm, and a trail of dye

could be traced back along the ventral and lateral brain surfaces to the CN. Weak staining of blood vessels, as in Group 2, was also seen. Statistical comparison of the hyperthermic responses produced by PGE_1 in the three groups revealed no significant difference in latency or ΔT_c. For FI_{180}, only one comparison was significant; the value for Group 1 was larger than that for Group 2 (0.98°C-hr versus 0.52°C-hr).

The circles on the series of frontal sections presented in Fig. 2 denote the approximate location of the CN injection sites for the rats included in Table 1. With one exception, the injections were within or just medial to the dorsal or ventral cochlear nucleus. In one rat with an injection site outside the cochlear nuclei, it was noted that dye administered into the injection site (flocculus of the cerebellum) had diffused extensively into the dorsal cochlear nucleus. In the figure, the injection sites are coded with respect to the intensity of the response elicited by PGE_1. There was no obvious relationship between the response magnitudes and the anterior-posterior plane or the medio-lateral position of the injection sites. The squares shown in Fig. 2 represent 4 additional sites that differed distinctly from the others in that PGE_1 injections into these loci produced hyperthermia only after a long latency (8.13±1.20 min for these 4 sites versus 0.53±0.25 min for the other 19 sites). No explanation based on site position or microscopic appearance could be found for the difference in latencies.

CN Injections of ^{3}H-PGE_1

The results obtained from the ^{3}H-PGE_1 diffusion study are presented in Table 2. With one exception, less than 1% of the injected radiolabel was found in the tissue blocks containing the AH/PO. In 3 rats, all of which responded with a relatively large hyperthermia when injected with unlabeled PGE_1, no radiolabel was detected within the AH/PO. In those rats which had detectable amounts of tritium in the AH/PO tissue blocks, there was a consistent predominance of counts in the block ipsilateral to the injection site (ipsilateral/contralateral ratio=1.1–2.4). The data were examined for a correlation between the ΔT_c or FI_{180} values associated with CN injection of unlabeled PGE_1 and the total tritium content of the tissue blocks containing the AH/PO. The Spearman Rank Correlation Coefficients (r_s) of −0.16 and −0.13, respectively, were not significant ($p>0.05$, Kendall test). There was also no correlation between ΔT_c or FI_{180} and the tritium content of the AH/PO block ipsilateral to the ^{3}H-PGE_1 injection site ($r_s=-0.46$ and −0.13, $p>0.05$). Examination of frozen sections of the posterior part of the brain in each rat revealed that the injection sites were located in the cochlear nucleus complex between anterior-posterior planes −2.8 and −3.6.

Effect of AH/PO Lesions on the Hyperthermic Response to PGE_1 Injected in the CN, Third Cerebral Ventricle and Interpeduncular Cistern

To ascertain whether the AH/PO region is essential for the mediation of the hyperthermic response produced by PGE_1 injected at the CN, the AH/PO was destroyed bilaterally prior to PGE_1 injection in 9 rats. Lesions of a size and position which resulted in the destruction of all rostrally situated prostaglandin sensitive tissue were employed. The adequacy of the lesions in this regard was demonstrated by their ability to abolish the hyperthermic effect of 100 ng of PGE_1 injected into the third ventricle or the interpeduncular cistern (Table 3). Such lesions also abolished the hyperthermic effect of PGE_1 injected at the CN (Table 3). That the injections had been made into the CN complex was confirmed by examination of frozen frontal sections of the brain of each rat. The AH/PO lesions were large, oblate spheroids which destroyed bilaterally all anterior hypothalamic and medial and lateral preoptic tissue. They extended laterally as far as 2.5 mm from the midline and as far dorsally as the medial septal nuclei, with these being destroyed in many animals. Rostrally, the lesions extended through the diagonal band to anterior-posterior plane 9.4. The maximum caudad extent was to the level of the ventromedial hypothalamic nuclei (AP 6.0). The posterior hypothalamus was not damaged. In preliminary experiments, smaller lesions which produced a more selective destruction of AH/PO tissue were found to produce only a modest reduction in the hyperthermic response to PGE_1 injected into the third ventricle or interpeduncular cistern. In spite of the large size of the lesions, all 9 rats used in this experiment were capable of maintaining core temperature at a level not lower than 35.5°C throughout the four-hour session.

DISCUSSION

The results demonstrate clearly that unilateral injection of 100 ng of PGE_1 into or near the cochlear nuclei reliably produces a core temperature increase. Thirty of 33 rats so injected (Table 2 and Fig. 2) experienced a temperature increase of at least 0.5°C (mean=0.83°C) which usually started within the first minute after the injection. However, the results regarding where PGE_1 acted to produce this effect are less definitive. Although some of our findings suggest a local action at or near the CN, others implicate an action upon AH/PO tissue.

In support of a possible action on the AH/PO are some of the findings of the dye diffusion and ^{3}H-PGE_1 diffusion studies. In 12 of 19 animals in which dye was injected into the CN, evidence of transport to near the ventral surface of the AH/PO within 10 min after injection was found. In only 5 rats was staining of brain tissue which clearly indicated bulk transport of dye in the subarachnoid CSF detected. However, in all 12 rats, the blood vessels on the ventral aspect of the brain had a weak bluish cast. The basis for the preferential staining of the vessels is unknown, but regardless of the mechanism, dye (or PGE_1) in the vessels or in the perivascular spaces would have ready access to the parenchyma of the AH/PO [2,11].

The ^{3}H-PGE_1 diffusion study confirmed that PGE_1 injected at CN can rapidly reach AH/PO tissue. In 7 of 10 rats, counts representing 0.071 to 1.39 ng of unlabeled PGE_1 were found in the AH/PO tissue blocks at 2 min after injection. Although this is only a minute fraction of the injected amount, the great sensitivity of the AH/PO to PGE_1 must be taken into account. Veale and Whishaw [12], for example, reported that a total dose of 0.2 ng of PGE_1 injected bilaterally into the AH/PO of the rat produced a significant body temperature elevation. However, in the study of Veale and Whishaw, a concentrated solution of PGE_1 was applied to a small volume of AH/PO tissue, whereas in our experiments the distribution of PGE_1 must have been much more diffuse. Thus, it is not certain that the PGE_1 which reached the AH/PO tissue contributed importantly to the hyperthermic response.

Findings which suggest that PGE_1 injected into the CN acted locally to produce hyperthermia are the lack of correlation between hyperthermic response and dye or ^{3}H-PGE_1

distribution to the AH/PO. Seven of 19 rats injected with dye at CN exhibited no dye distribution beyond CN, yet, with respect to latency and ΔT_c, the PGE_1-induced hyperthermia of this group did not differ significantly from that of rats in which dye was seen to stain the brain tissue and/or blood vessels near the AH/PO. In the ^3H-PGE_1 study, 3 rats in which no ^3H-PGE_1-derived counts were found in the AH/PO blocks experienced substantial temperature increases when injected at the CN with unlabeled PGE_1. Also, there was no statistically significant correlation between the tritium content of the AH/PO blocks in the 10 rats involved in the study and the magnitude of hyperthermia elicited by unlabeled PGE_1.

The final major finding of this study, that destruction of the AH/PO abolished the response to PGE_1 injected into CN, is consistent with either a local or an AH/PO site of action. Obviously, such a result could be due to the destruction of PGE_1-sensitive tissue in the AH/PO. However, it is also possible that neuronal pathways passing through or terminating in the destroyed area are necessary for mediation of the hyperthermic effect. Another possible explanation consistent with a local action is that massive rostral diencephalic lesions result in the loss of tonic descending inhibition of midbrain structures which, in turn, inhibit lower brainstem pathways involved in thermogenesis [1]. The ultimate effect, enhanced inhibition at the lower brainstem, may have prevented PGE_1 acting at CN from eliciting a termperature increase.

Although an unequivocal conclusion regarding the site of action of PGE_1 injected at CN cannot be drawn from the data discussed above, the only interpretation which is consistent with all the available evidence is that, although a minute amount of PGE_1 sometimes does reach the AH/PO following an injection at CN, PGE_1 is not importantly involved in the hyperthermic response produced by the injection. Additional experiments will be required to validate this conclusion. However, some ancillary support is provided by previous studies in which 100–200 ng doses of PGE_1 were injected at numerous upper and lower brainstem sites which bordered on the subarachnoid space [8,15]. Many of these sites were much closer to the AH/PO than CN, yet none of the injections produced a hyperthermic effect. Thus, it seems clear that there is something unusual about the CN region. It is either an extra-AH/PO site of prostaglandin action as postulated or it provides a unique access portal to AH/PO tissue of prostaglandins released in the CN region and contiguous tissue and/or present in the CSF near the acoustic tubercle.

An impediment to the acceptance of the cochlear nuclei as a site of action for PGE_1 is that these auditory relay nuclei have no recognized involvement in fever production or the control of body temperature. However, it is known that there is a convergence of thermal and non-thermal sensory input within the brainstem reticular formation [7], that there are projections from the cochlear nuclei to reticular neurons [13] and that there exist pathways from the reticular formation to preoptic thermosensitive units [5]. It is thus possible that the temperature increase produced by CN injection of PGE_1 is due to enhanced ascending activity in the latter pathways initiated by a false auditory input. It can also be speculated that PGE_1 injected at CN is not acting upon the cochlear nuclei but, rather, on a superficial chemosensitive zone analogous to the ventromedullary zones which have been implicated in cardiovascular and respiratory regulation [11,14] or on a nearby structure whose involvement in thermoregulation has yet to be recognized.

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