Preoptic α₁- and α₂-noradrenergic agonists induce, respectively, PGE₂-independent and PGE₂-dependent hyperthermic responses in guinea pigs

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Feleder, Carlos, Vit Perlik, and Clark M. Blatteis. Preoptic α₁- and α₂-noradrenergic agonists induce, respectively, PGE₂-independent and PGE₂-dependent hyperthermic responses in guinea pigs. Am J Physiol Regul Integr Comp Physiol 286: R1156–R1166, 2004.—We have shown previously that norepinephrine (NE) microdialyzed into the preoptic area (POA) of conscious guinea pigs stimulates local PGE₂ release. To identify the cyclooxygenase (COX) isozyme that catalyzes the production of this PGE₂ and the adrenoceptor (AR) subtype that mediates this effect, we microdialyzed for 6 h NE, cirazoline (α₂-AR agonist), and clonidine (α₂-AR agonist) into the POA of conscious guinea pigs pretreated intrapeptically (intra-POA) with SC-560 (COX-1 inhibitor) or nimesulide or MK-0663 (COX-2 inhibitors) and measured the animals’ core temperature (Tc) and intra-POA PGE₂ responses. Cirazoline induced Tc rises promptly after the onset of its dialysis without altering PGE₂ levels. NE and clonidine caused early falls followed by late rises of Tc; intra-POA PGE₂ levels were closely correlated with this thermal course. COX-1 inhibition attenuated the clonidine-induced Tc and PGE₂ falls but not the NE-elicted hyperthermia, but COX-2 inhibition suppressed both the clonidine- and NE-induced Tc and PGE₂ rises. Coinfused cirazoline and clonidine reproduced the late Tc rise of clonidine but not its early fall and also not the early rise produced by cirazoline; on the other hand, the PGE₂ responses were similar to those to NE. Prazosin (α₁-AR antagonist) and yohimbine (α₂-AR antagonist) blocked the effects of their respective agonists. These results indicate that α₁- and α₂-AR agonists microdialyzed into the POA of conscious guinea pigs evoke distinct Tc responses: α₁-AR activation produces quick, PGE₂-independent Tc rises, and α₂-AR stimulation causes an earlier Tc rise and a late, COX-2/PGE₂-dependent Tc rise.

thermoregulation; cyclooxygenase inhibitors; prostaglandin E₂; noradrenergic agonists and antagonists; core temperature; norepinephrine

There is thus no consensus as yet as to how NE and PGE₂ may interact in the POA during fever. The issue has relevance to current hypotheses of the mechanism of fever induction. Thus it is well documented that peripheral pyrogens activate noradrenergic pathways in the brain (14, 26) and that NE is released in the POA in conjunction with the febrile response (31, 38). The involvement of PGE₂ in fever production is now all but axiomatic, but the dynamics and source of the pyrogen-induced elevation of preoptic PGE₂ levels are still controversial (5). If NE were the direct stimulus for its increased production, it might be expected from the preceding that it would begin promptly and that, in turn, the elevated PGE₂ levels would inhibit the further release of NE. Furthermore, to support the hypothesis of such an interaction in fever production, it would be necessary to show that the induction of PGE₂ by NE is mediated in the POA by COX-2, since it is now generally agreed that COX-2 is the isoform of the enzyme that catalyzes the production of PGE₂ in response to pyrogenic stimuli (29, 48, 64, 77).

The present study was undertaken, therefore, to test the hypothesis that COX-2 is the isoform that mediates the NE-enhanced expression of PGE₂ in the POA and to identify the adrenoceptor (AR) subtype(s) that may be involved in this effect. Because physiological stimuli cause NE to be released from its neuronal terminals not in a single burst but in repetitive pulses over a varying time until its stores become exhausted (2, 65) and because the repetitive pattern of delivery is better approximated by microdialysis, when NE is presented to the tissue over a longer duration, than by microinjection, when it is presented acutely in a microdroplet (47), we microdialyzed rather than microinjected NE and specific α₁- and α₂-AR

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agonists and antagonists into the POA of conscious guinea pigs pretreated intraperitoneally (intra-POA) with selective COX-1 and COX-2 inhibitors and measured the animals’ Tc and POA PGE2 levels. Our results validate the hypothesis and reveal additionally that the positive AR agonists of NE exert different effects. The activation of α2-ARs evokes very promptly a Tc rise with no associated PGE2 release, whereas the activation of α2-ARs induces an initial Tc fall followed later by a Tc rise, the upregulation of COX-2 and the consequent production of PGE2. However, when administered together (to mimic NE), the hyperthermic effect of α1-AR stimulation is nullified by the initial hypothermic effect of the simultaneous activation of α2-ARs. COX-1 does not appear to have a demonstrable role in the Tc rise caused by NE under these conditions but may be involved in the α2-AR-mediated Tc fall.

MATERIALS AND METHODS

Animals

Male, pathogen-free, Hartley guinea pigs (301–350 g; Charles River Laboratories, Wilmington, MA) were used in these experiments. The animals were quarantined for 1 wk, three to a cage, before any experimental use. Tap water and food (Agway Prolab guinea pig diet) were available ad libitum. The ambient temperature (Ta) in the animal room was 23 ± 1°C; light and darkness alternated, with light on from 0600 to 1800. After quarantine, to moderate the psychological stress associated with the experiments, the animals were trained to the experimental procedure for 1 wk (daily for 4 h) by handling and placement in individual, locally fabricated, semicircular wire mesh confiners designed to prevent their turning around and to minimize their forward and backward movements, but without causing restraint stress; rodents readily adapt to such confinement and show no sign of discomfort or anxiety (56). All animal protocols were approved by the University of Tennessee Health Science Center Animal Care and Use Committee and fully conform with the standards established by the US Animal Welfare Act and by the documents entitled “Guiding Principles for Research Involving Animals and Human Beings” (1).

Surgical Procedure

All the animals received a prophylactic injection of the antibiotic gentamicin sulfate (6 mg/kg im) immediately before surgery and once a day for the following 2 days. Microdialysis guide cannulas were implanted by methods described previously (46, 47). Briefly, the guinea pigs were anesthetized with ketamine-xylazine (35–5 mg/kg im). Under aseptic conditions, a sterile, 17-mm-long, 17-gauge, thin-walled stainless steel guide cannula with a tightly fitting indwelling styllet was implanted stereotaxically into the left medial POA [antero-posterior = 11.6 mm, lateral = 1.0 mm, ventral = −9.0 mm, according to the atlas of Laparelo (32)] and fixed to the skull with tissue adhesive and immediately perfused with sterile aCSF via sterile 1-ml tuberculin syringes clamped to a syringe pump (model A-99; Razel Scientific Instruments, Stamford, CT) as the driver; to run six animals simultaneously, the pushers of two pumps were modified so that each could accommodate three syringes at a time. The flow rate of the perfusion was 2 μl/min (46). A 90-min stabilization period preceded all the experimental treatments.

After an experiment, the guinea pigs were euthanized by isoﬂurane overdose, and the brains were quickly removed and stored in 10% phosphate-buffered Formalin for later histological verification of the placement of the dialysis probe tips. Localization of the center of the dialysis probe within 0.5 mm of the medial POA was regarded as the correct placement (Fig. 1). Only the data from animals with confirmed preoptic placement of the probes are included in this report.

Physiological Measurements

Seven days after surgery, the guinea pigs, fully conscious, were loosely restrained in their individual wire mesh confiners at Tc 23 ± 1°C. The Tc of the guinea pigs were monitored constantly and recorded at 2-min intervals for the duration of an experiment on a Macintosh Plus 1Mb microcomputer through an analog-to-digital converter, using precalibrated copper-constantan thermocouples inserted 5 cm into the colon. The data were displayed digitally on a monitor, printed on an ImageWriter printer, and stored on a diskette. The effluent from the microdialysate probes were collected over 30-min intervals continuously throughout the experiments. The PGE2 content of the samples was evaluated using a PGE2 enzyme immunoassay kit (no. 931–001; Assay Designs, Ann Arbor, MI). To
obviate possible effects of circadian variations, all the experiments were begun at the same time of day (0830).

Experimental Designs

Experiment 1: NE effects on \( T_c \). To determine the thermal effect of intra-POA NE and to identify the \( \alpha \)-AR subtype(s) that may mediate this effect, we microdialyzed NE, cirazoline, clonidine, prazosin, or yohimbine into the POA of conscious guinea pigs for 6 h, or prazosin for 1 h followed by cirazoline for 5 h, or yohimbine for 1 h followed by clonidine for 5 h. \( T_c \) and preoptic PGE\(_2\) levels were measured as described earlier. The 6-h total duration of this procedure was chosen to align it with the temporal course of a prototypic endotoxemic fever in conscious guinea pigs (60, 64).

Experiment 2: \( \alpha_2 \)-ARs and COX. Because clonidine, but not cirazoline, induced \( T_c \)-associated POA PGE\(_2\) level changes (see results), to identify the COX isozyme presumptively involved in these effects, we microdialyzed SC-560, nimesulide, or MK-0663 for 1 h into the POA of conscious guinea pigs and clonidine for the following 5 h. \( T_c \) and preoptic PGE\(_2\) levels were determined as before. The effects of these COX inhibitors on \( T_c \) and PGE\(_2\) were also tested.

Experiment 3: validation of preoptic NE effects. To relate the results of experiments 1 and 2 to the actions of intra-POA NE, we microdialyzed for 1 h prazosin, yohimbine, or MK-0663 into the POA of conscious guinea pigs, followed for 5 h by NE. \( T_c \) and preoptic PGE\(_2\) levels were monitored as above.

Finally, to correlate the effects of NE to those evoked by the \( \alpha_1 \)-AR and \( \alpha_2 \)-AR agonists, but without the confounding effects of 0.1% ascorbate (low pH and antioxidant vehicle), we microdialyzed cirazoline and clonidine combined into a single solution intra-POA for 6 h. \( T_c \) and preoptic PGE\(_2\) levels were monitored as above.

Statistical Analyses

The results are reported here as means ± SE. The values of \( T_c \) are changes from basal values [initial \( T_c \) (\( T_{ci} \), the \( T_c \) at 2-min intervals averaged over the last 10 min of the preceding 90-min stabilization period] plotted at 6-min intervals. Latencies of \( T_c \) changes were defined as the intervals (in min) between the onset of a treatment (0 or 60 min) and the first \( T_c \) rise or fall greater than 0.2°C (i.e., the SD of the \( T_c \) of aCSF-treated guinea pigs) that continued uninterrupted beyond ±0.5°C. The PGE\(_2\) data are expressed as changes relative to their values before a treatment (\( P_0 \)). A repeated-measures ANOVA was used to compare the \( T_c \) and PGE\(_2\) changes between groups; factor 1 was the between-groups factor (the treatment), and factor 2 was the within-subjects factor (the different sampling periods), followed, if significant differences were found, by a point-by-point Tukey-Kramer multiple comparison test. The analyses were performed using Instat 3 (Graph Pad Software; Instant Biostatistics, San Diego, CA). Each variable was considered to be independent. The 5% level of probability was accepted as statistically significant.

RESULTS

Experiment 1: NE Effects on \( T_c \)

The intra-POA microdialysis of aCSF per se did not evoke any \( T_c \) and intra-POA PGE\(_2\) changes (Fig. 2, A and B). In contrast, aCSF + 0.1% ascorbic acid, the NE antioxidant, caused a prompt, statistically significant approximately −0.6°C decrease in \( T_c \) (latency: 45 ± 12 min); it reached its nadir in 120 min and then returned to its basal level by ~120 min. The levels of PGE\(_2\) in the microdialysate effluents, however, were not changed by this treatment (Fig. 2, C and D). NE + 0.1% ascorbic acid induced a biphasic thermal response. The first, a \( T_c \) fall, coincided in magnitude with that induced by ascorbic acid but lasted longer. It was followed by a second response, a \( T_c \) rise, that gradually began after ~120 min and peaked ~0.8°C above \( T_{ci} \), a statistically significant increase: this rise was maintained until the end of the experiment. Preoptic PGE\(_2\) levels increased significantly in these guinea pigs in approximate temporal correspondence with their \( T_c \) rises (Fig. 2, E and F).

The microdialysis of cirazoline evoked a statistically significant \( T_c \) rise very quickly after the onset of its administration (latency: 15 ± 6 min). The maximum (~1°C) was attained in ~150 min, continued unaltered for ~2 h, and then gradually declined even though the perfusion was continuing. PGE\(_2\) levels, however, were not altered (Fig. 2, A and B). Prazosin perfused over the full 6 h of the experiment altered neither the \( T_c \) nor the PGE\(_2\) levels of the guinea pigs (Fig. 3, A and B), but prazosin administered for 1 h before cirazoline completely abolished the effect of cirazoline on \( T_c \); PGE\(_2\) was not affected (Fig. 3, E and F).

Clonidine induced a gradual, significant \( T_c \) fall followed by a rapid, significant \( T_c \) rise compared with aCSF alone (Fig. 2A). The \( T_c \) decline began almost immediately after the onset of the dialysis (latency: 15 ± 6 min) and reached approximately −0.8°C in ~160 min. It then increased progressively until the end of the experiment to ~1°C above \( T_{ci} \). Intra-POA PGE\(_2\) levels changed significantly in close correlation with the \( T_c \) changes (Fig. 4, A and B). The perfusion of yohimbine over the 6-h duration of the experiment affected neither the \( T_c \) nor the PGE\(_2\) levels of the guinea pigs (Fig. 4, C and D), but yohimbine administered for 1 h before clonidine completely abolished both the \( T_c \) and PGE\(_2\) falls and rises caused by clonidine alone (Fig. 4, E and F).

Experiment 2: \( \alpha_2 \)-ARs and COX

The microdialysis of SC-560 for 1 h before that of clonidine did not alter the late effects of clonidine on \( T_c \) and intra-POA PGE\(_2\) levels. However, it inhibited its early effects on these variables. PGE\(_2\) levels began to increase significantly ~150 min after the onset of clonidine, rising continuously until the end of the experiment, in coincidence with \( T_c \) (Fig. 5, A and B). In contrast, the microdialysis of nimesulide or MK-0663 blocked the late rises of both \( T_c \) and PGE\(_2\) evoked by the

Fig. 1. Photomicrograph of a coronal section through the forebrain of a guinea pig, showing the track of a microdialysis probe guide cannula implanted into the preoptic area (POA). The guide cannulas were placed 7 days before an experiment (see materials and methods for details). AC, anterior commissure; 3V, third ventricle; OC, optic chiasma.
microdialysis of clonidine alone; indeed, MK-0663 inverted the T<sub>c</sub> rise into a significant fall, but neither had an effect on the early responses. PGE<sub>2</sub> levels changed significantly in close temporal correlation with these thermal courses (Fig. 5, C–F). None of the COX inhibitors or their vehicle, DMSO, microdialyzed for 6 h, significantly affected the T<sub>c</sub> or the intra-POA PGE<sub>2</sub> levels of the guinea pigs (not illustrated).

**Experiment 3: Validation of Preoptic NE Effects**

The intra-POA microdialysis of prazosin for 1 h before that of NE attenuated the initial T<sub>c</sub> fall caused by NE alone but did not alter the late rises of T<sub>c</sub> and intra-POA PGE<sub>2</sub> levels induced by NE (Fig. 2, E and F). Thus the T<sub>c</sub> increased gradually, beginning ~180 min after the start of the perfusion of NE and
reaching a significant elevation of \(\sim 1^\circ C\) by the end of the experiment. PGE2 levels again changed in close correlation with the thermal courses (Fig. 6, A and B). Yohimbine microdialyzed intra-POA for 1 h before NE also prevented the early fall of \(T_c\) and the late rises of \(T_c\) and PGE2 levels induced by NE. Similarly, MK-0663 completely abolished the \(T_c\) and PGE2 level changes evoked by NE. Indeed, MK-0663 inverted both the \(T_c\) and PGE2 late rises into falls, in close correlation with each other (Fig. 6, C-F).

The initial \(T_c\) falls produced by both NE (Fig. 2E) and clonidine (Fig. 4A) were abrogated when cirazoline and clonidine were microdialyzed together (Fig. 7A). However, the late \(T_c\) rises induced by both NE and clonidine were still evident. Thus \(T_c\) increased progressively from \(-200\) min after the onset of the perfusion until the end of the experiment (to \(\sim 1.2^\circ C\) above \(T_{ci}\)). Preoptic PGE2 levels, on the other hand, decreased significantly promptly after the onset of the experimental treatment and remained low for \(-180\) min and then

![Fig. 4. Effects on \(T_c\) and preoptic PGE2 levels, respectively, of the \(\alpha_2\)-AR agonist clonidine (A and B), the \(\alpha_2\)-AR antagonist yohimbine (C and D), and yohimbine + clonidine (E and F) microdialyzed into the POA of conscious guinea pigs. Abbreviations and conventions as in Fig. 2. *P < 0.05.](image)

![Fig. 5. Effects on \(T_c\) and preoptic PGE2 levels, respectively, of the COX-1 inhibitor SC-560 + clonidine (A and B) and the COX-2 inhibitors nimesulide + clonidine (C and D) and MK-0663 (E and F) + clonidine microdialyzed into the POA of conscious guinea pigs. Abbreviations and conventions as in Fig. 2. *P < 0.05.](image)
increased in correlation with the late $T_c$ rise (Fig. 7B), analogously to the full response to clonidine (Fig. 4B) and the late response to NE (Fig. 2F).

**DISCUSSION**

The selective $\alpha_1$-AR agonist cirazoline induced in this study prompt $T_c$ rises without affecting basal preoptic PGE$_2$ levels, whereas the selective $\alpha_2$-AR agonist clonidine caused early $T_c$ falls and delayed $T_c$ rises, both associated with parallel changes in the levels of POA PGE$_2$. The thermal effects of both these agonists were validated by their blockade by their respective antagonists, prazosin and yohimbine. Furthermore, both the increases in $T_c$ and POA PGE$_2$ levels caused by clonidine were prevented by the intra-POA microdialysis of the COX-2 inhibitors nimesulide and MK-0663, but only the initial decreases of these variables were suppressed by that of the COX-1 inhibitor SC-560. (Because the hyperthermic effect of cirazoline was not accompanied by changes in the levels of POA PGE$_2$, the effects of the COX inhibitors were not tested in its regard.) The intra-POA microdialysis of NE reproduced the early and the late $T_c$ and preoptic PGE$_2$ level changes induced by clonidine but not the early $T_c$ rise evoked by cirazoline; both the clonidine-mediated effects were inhibited by yohimbine and MK-0663 but not by prazosin. Cirazoline and clonidine microdialyzed together replicated the late $T_c$ rises but not the early $T_c$ falls elicited by clonidine and NE and also not the early $T_c$ rises caused by cirazoline; on the other hand, it induced both the POA PGE$_2$ falls and rises associated with clonidine and NE. The present results thus indicate that $\alpha$-AR agonists microdialyzed into the POA of conscious guinea pigs evoke differentially mediated $T_c$ responses. To the best of our knowledge, they are also the first demonstration in the nervous system that the induction of postsynaptic PGE$_2$ by presynaptic NE is mediated by $\alpha_2$-ARs and modulated by COX-2.

![Fig. 6. Effects on $T_c$ and preoptic PGE$_2$ levels, respectively, of the $\alpha_1$-AR antagonist prazosin + NE (A and B), the $\alpha_2$-AR antagonist yohimbine + NE (C and D), and the COX-2 inhibitor MK-0663 + NE (E and F) microdialyzed into the POA of conscious guinea pigs. Abbreviations and conventions as in Fig. 2. *$P < 0.05$.](http://ajpregu.physiology.org/)

![Fig. 7. Effects on $T_c$ (A) and preoptic PGE$_2$ levels (B) of the $\alpha_1$-AR agonist cirazoline + the $\alpha_2$-AR agonist clonidine microdialyzed together into the POA of conscious guinea pigs. Abbreviations and conventions as in Fig. 2. *$P < 0.05$.](http://ajpregu.physiology.org/)
Although the presence of $\alpha_1$-ARs on thermosensitive neurons in the POA has been demonstrated previously by various means (35, 37), the thermal responses of different species to intra-POA microinjected $\alpha_1$-AR agonists have generally been inconsistent (9, 10). Indeed, we found in a previous study (50) that the $\alpha_1$-AR agonist methoxamine microdialyzed into the POA of conscious guinea pigs for 3 h had no thermal effect. The very prompt hyperthermic response to the specific $\alpha_1$-AR agonist cirazoline observed in this study was therefore unexpected. We have no explanation at this time for the discrepant responses to the two drugs other than to speculate that they may be accounted for by the lower potency of methoxamine compared with cirazoline (8, 63). The possibility that cirazoline may have leaked out of the central nervous system and acted on peripheral vascular $\alpha_1$-ARs, causing cutaneous vasoconstriction, seems remote because the dose that was microdialyzed intra-POA in this study was effectively ~1,000 times lower than its minimally effective vasoconstrictive dose (18, 37). It is also possible that the observed $T_c$ rises may have been caused by a stimulatory action of cirazoline not mediated by $\alpha_1$-ARs, but this too is unlikely since its hyperthermic effect was inhibited by the specific $\alpha_1$-AR antagonist prazosin (Fig. 3). Significantly, the cirazoline-induced hyperthermia occurred in this instance without any associated changes in the levels of intra-POA PGE$_2$; i.e., it was evoked quickly and without the intermedation of PGE$_2$. This finding is novel and suggests that its mode of action on neurons is direct. We propose therefore (Fig. 8) that the activation of $\alpha_1$-ARs on postsynaptic warm-sensitive or thermosensitive neurons directly reduces or augments, respectively, the activities of these neurons, both responses promoting heat conservation according to the models of Hammel (19) and Boulant (7). Since these neurons, moreover, are thought to inhibit synaptically connected cold-sensitive neurons, these are concomitantly facilitated, stimulating heat production (not illustrated). The combination of these effector mechanisms raises $T_c$. The eventual decline of the hyperthermic action of cirazoline observed in the present study was presumably due to the desensitization of the relevant postsynaptic neurons, since prolonged exposure to a transmitter can gradually attenuate the responsiveness of neurons that originally were activated by it. The specific $\alpha_1$-AR subclass involved in this hyperthermic effect remains to be identified; Mallick et al. (34) have reported that the NE-stimulated increase in sodium pump activity of rat brain homogenates is mediated by $\alpha_{1A}$-ARs. Based on the foregoing, the present results would imply that NE released endogenously in the POA should exert a prompt hyperthermic action mediated by quickly activated postsynaptic $\alpha_1$-ARs. A discrepancy, however, appears to exist: the presence of NE, as mimicked by its intra-POA microdialysis, induced a $T_c$ fall rather than a $T_c$ rise; it will be addressed later.

The intra-POA microdialysis of the $\alpha_2$-AR agonist clonidine evoked in this study a biphasic thermal response, viz., an initial, early decrease in $T_c$, followed by a late, extended $T_c$ rise. Clonidine has consistently been reported to evoke $T_c$ falls when microinjected intra-POA in various species (39, 55). In our own previous studies (50, 51), it also induced dose-dependent $T_c$ falls in conscious guinea pigs when microdialyzed intra-POA for 3 h at doses from 0.5 to 10 $\mu$g/$\mu$L. It did so again in the present study, at 2 $\mu$g/$\mu$L (Fig. 4). $\alpha_2$-ARs occur on both presynaptic and postsynaptic neurons. The identity of their subclasses specifically involved in this effect was not investigated in the present study; in mice, both $\alpha_{2A}$- and $\alpha_{2C}$-ARs have been implicated in the hyperthermic action of clonidine (24, 28, 58).

In the present study, this hypothermic response was accompanied, moreover, by a concurrent decrease in the animals’ preoptic PGE$_2$ levels, a new finding. Like the $T_c$ fall, it was blocked by pretreatment with the selective $\alpha_2$-AR antagonist yohimbine. Unexpectedly, both the $T_c$ and PGE$_2$ falls were also prevented by pretreatment with the COX-1 inhibitor SC-560 (Fig. 5). At least two possibilities could account for the latter surprising findings. Both tentatively involve the second messenger cAMP. Thus 1) various lines of evidence have indicated that NE induces the production of cAMP in first-order neurons in hypothalamic tissue slices (17); it is controversial, however, whether it does so by stimulating $\alpha_1$- or $\alpha_2$-ARs (52, 59). It was also reported long ago (11, 12) that the intra-POA microinjection of its analog agonist, dibutyryl-cAMP, into rabbits and rats causes a rapid fall followed by a prolonged rise in $T_c$ and that the latter rise is blocked by acetaminophen, then a nonspecific COX inhibitor, but lately a putatively specific inhibitor of the presumptive COX-1 variant, COX-3 (61). This late rise was attributed to PGE$_2$ generated in consequence of tissue damage associated with the microinjection procedure per se. Finally in this regard, it has also been...
shown that cAMP increases the activity of rat preoptic warm-sensitive neurons in slice preparations (7), an effect that would promote heat loss and, consequently, a Tc fall (7, 19). Taken together, these data infer that the clonidine-induced fall in Tc observed in the present study could have been due to the hypothermic action of cAMP released by α2-ARs (since the effect was blocked by yohimbine pretreatment). This explanation is insufficient, however, to also account for the decrease in preoptic PGE2 that accompanied this Tc fall. Hence, 2) it could be that the stimulation by clonidine of postsynaptic α2-ARs also rapidly activates COX-1 (since the effect was blocked by SC-560) and induces the preferential production of PGD2. cAMP is a well-known activator of COX expression (44), and, from a strictly stoichiometric point of view, if under these conditions PGD synthase were activated relative to (presumably cytosolic) PGE synthase, one would expect less PGE2 and more PGD2 to be produced; both synthases are downstream of COX-1 and would be competing for the same, initially limited substrate, arachidonic acid. PGD2 has been shown to be hypothermic in various species (69), including guinea pigs (4), and it is also more abundant in brain than PGE2 (20). COX-1 is expressed constitutively in most cells, including neurons, and its PG products mediate basic physiological functions in normal tissue; the involvement of COX-1-dependent PGD2 in the observed effects is, therefore, quite plausible. Indeed, its blockade would prevent them, as was observed in this study. COX-1 blockade, however, does not affect the basal level of preoptic PGE2 (not illustrated).

The continued microdialysis of clonidine converted the initial Tc and preoptic PGE2 falls into concurrent, protracted increases (Fig. 4). Because both these rises were also inhibited by yohimbine pretreatment, they too were evidently α2-AR mediated; and because, moreover, they were prevented by the prior microdialysis of the selective COX-2 inhibitors nimesulide and MK-0663, this late hypothermic response was mediated by COX-2-dependent PGE2. The production of PGE2 by NE is well documented (23, 33, 60), but the AR subtype and COX isofrom that mediate PGE2 production were not identified (45). The present data thus add the information that this response is α2-AR mediated and specifically catalyzed by COX-2. COX-2 is expressed constitutively in brain cells, and various studies have demonstrated that, although both COX-1 and COX-2 enzymes are upregulated by various inflammatory stimuli, COX-2 is the more readily inducible isofrom (72). Its induction in this study could have been initiated by cAMP simultaneously with its activation of COX-1. One to 4 h are required for new gene expression and translation of COX-2 in LPS-activated phagocytic, endothelial, and other relevant cells ex vivo and in vivo (25, 48, 77), a duration that coincides with the interval between the onset of the microdialysis of clonidine and NE and the beginning of the rises of Tc and the levels of POA PGE2 induced by both these agonists in the present study (Figs. 2 and 4). The involvement of COX-2 under the present experimental conditions is therefore also concordant. In support, the late hypothermic response to clonidine microinjected intracerebroventricularly into conscious mice is suppressed in COX-2 gene-ablated animals (unpublished observation). Although we have previously observed a biphasic hypo/hypothermic response to intra-POA microinjected (rather than microdialyzed) clonidine (55), in that study in contrast to the present one, the α2-AR antagonist rauwolscine microinjected 10 min before clonidine abolished the hypothermia without affecting the subsequent hyperthermia; the latter was attenuated by the intramuscular injection of the nonspecific COX inhibitor indomethacin 20 min after the intra-POA microinjection of clonidine. This response was similar to that to dibutyryl-cAMP reported by Dascombe (11, 12). We interpreted those data as verifying the α2-AR-mediated hypothermic action of clonidine and, like Dascombe, attributed the subsequent Tc rise to contamination of the thermal response to this agonist by PGE2 released in the POA consequent to the acute inflammatory response to the microinjection procedure per se (57). However, in view of the present findings, we now suggest that the late reduced hypothermic response to microinjected clonidine observed in that previous study (55) was also partly accomplished by α2-AR-mediated, as contrasted only to injury-induced, upregulation of COX-2-dependent PGE2.

The brain cell type(s) expressing COX-2 in the present instance was not investigated, but the increase of COX-2 after, for example, the peripheral administration of LPS is observed in astrocytes, microglia, perivascular cells, and cerebrovascular endothelial cells but only irregularly in neurons (16, 29, 36, 48, 71); neurons, in any case, secrete only small amounts of PGE2 (22). Hence, we conjecture that the PGE2 collected in the microdialysate effluents from the POA extracellular space in the present experiments was generated by astrocytic processes contacting noradrenergic synaptic regions rather than by postsynaptic neurons (41). We propose therefore (Fig. 8) that the evoked release of NE by discharging presynaptic noradrenergic neurons stimulates astrocytic α2-ARs, generating arachidonic acid from membrane phospholipids; PGE2 is then formed by the action of COX-2 on the arachidonic acid. Which phospholipase (PL) forms are activated and how COX-2 and microsomal PGE synthase are upregulated under these conditions remain to be determined. In rat vascular smooth muscle cells, NE-stimulated postjunctional α2-ARs activate cytosolic PLAC2 [isoforms not identified (45)], but NE also binds to α2-ARs coupled to phosphoinositide-specific phospholipases (PI-PL) C and D (45). In rat hypothalami, LPS upregulates secretory PLAC2-IIA [cell types not identified (25)]. The mode of action of PGE2 on the activities of POA warm-sensitive and thermosensitive neurons is similar to that suggested earlier for α1-AR-mediated responses, viz., a reduction and an increase in firing rates, respectively (53). The PGE2-sensitive receptor involved in these neuronal effects may be the EP3 and/or EP4 subtypes; both have been linked to the development of fever and are present in the POA (15, 42, 43, 70, 76).

Although extensive, the data on the thermoregulatory actions of central NE are inconsistent, some indicating that NE reduces, others that it increases, and still others that it does not influence Tc (47, 49–51, 75). The discrepant results have been hard to reconcile because the methodologies, species, and doses employed by different investigators varied greatly. Indeed, we found, in common with other workers (66, 67, 75), that NE microinjected into the POA of conscious guinea pigs caused a rise in Tc but that, when microdialyzed, it evoked a hypothermic response (49–51). We attributed the Tc fall in the latter case to an artifact of the low pH (~3.5) of our perfusate and the neurotoxic effect of the potent antioxidant (sodium metabisulfite) present in that solution (Levophed). However,
that study, we discontinued the perfusion after 3 h and therefore missed its subsequent effects. The present finding that the intra-POA microdialysis of NE for 6 h induced a fall (as before) and then a rise in both Tc and POA PGE2 levels (analogous to the biphasic response to clonidine) and that both these effects were inhibited by yohimbine thus indicates that this biphasic response represents the authentic mode of action of NE microdialyzed for 6 h into the POA, i.e., that neither the observed falls nor the rises of these two variables were artifactual and that both events were mediated by α2-AR stimulation. On the other hand, the coadministration of cirazoline and clonidine, to mimic NE without its vehicle, 0.1% ascorbic acid, did not reproduce the initial NE-induced hypothermia although the fall in POA PGE2 was still evident. We ascribe the absence of a fall in Tc under these conditions to the observed, opposite thermal actions of these agonists, i.e., the direct, α1-AR-stimulated elevation of Tc by cirazoline was counteracted by the clonidine, α2-AR-mediated induction of, presumably, cAMP/PGD2 and the consequent depression of Tc. It is not clear, however, why then the α2-AR-mediated, hypothermic action of NE was not similarly antagonist by its α1-AR-mediated, hyperthermic effect, and why also the blockade of the latter effect by prazosin did not enhance the Tc fall. However, because the NE and combined cirazoline-clonidine solutions differed in this study only by the presence of ascorbic acid in the former, it is possible that the acidity of that additive accounted for the different effects. Indeed, the hypothermic effect of NE was larger than that of ascorbic acid yet smaller than that of clonidine. Because, furthermore, the NE- and clonidine-induced decreases in POA PGE2 levels were relatively similar, we speculate that pH could be more determinative of neuronal discharge activity (α1-AR) than of astrocytic signal transduction (α2-AR). Indeed, we found in an earlier study (60) that the intra-POA microdialysis of Levophed similarly abrogated the NE-induced changes in Tc but not those in PGE2.

Discounting, then, the possible, confounding effect of the NE solution and assuming that the observed responses to coadministered cirazoline and clonidine represent those of buffered NE, we conjecture that the hypothermic effect of endogenous NE released in the POA in response to a peripheral stimulus, in contrast to exogenous NE microdialyzed continuously into the POA, could be masked whereas its simultaneously activated hyperthermic effects could be manifested and occur in succession, i.e., the first (cirazoline) rapid in onset, α1-AR mediated and PGE2 independent, and the second (clonidine) delayed, α2-AR mediated and COX-2/PGE2 dependent. If existent, such a mechanism could be pertinent to a postulated mechanism of the febrile response to peripheral LPS that posits that the pyrogenic message may be transmitted to the brain by vagal afferents, ultimately arriving in the POA via ascending noradrenergic projections (6, 62, 73). To wit, the febrile response of guinea pigs to intravenous LPS is characteristically biphasic, and COX-2 plays a greater role in the late than in the early phase of the fever (64). If validated, this would implicate intra-POA NE pivotally in the modulation of the febrile response to LPS.

REFERENCES


