Acute dopamine/norepinephrine reuptake inhibition increases brain and core temperature in rats

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BRAIN CATECHOLAMINES ARE CONSIDERED to be involved in thermoregulation, especially in the preoptic area and anterior hypothalamus (PO/AH), which are the primary loci for maintenance of body temperature (2). A number of studies have investigated the relationship between dopamine (DA), norepinephrine (NE), and thermoregulation in the PO/AH (4, 7, 9, 32).

Our laboratory has recently shown that inhibition of PO/AH neurons by perfusion of tetrodotoxin (TTX) during exercise induces an additional increase in body core temperature (Tcore) with not only an enhancement of heat production responses but also an inhibition of heat loss mechanisms (10). The TTX-induced hyperthermia was not accompanied with any reduction in exercise behavior. Furthermore, our laboratory recently showed in a human study that central manipulation of the DA and NE system significantly influenced time trial performance, leading to a significantly higher Tcore (37). It is possible that this manipulation may enable an individual to dampen or override inhibitory signals arising from central nervous system (CNS) to cease exercise due to hyperthermia. This response appeared to occur without any change in the subjects’ perceived exertion or thermal sensation and may potentially increase the risk of developing heat illness (37). Therefore, it is interesting to examine the role of these neurotransmitter systems on thermoregulatory mechanisms.

Bupropion (Bup) is a dual dopaminergic and noradrenergic reuptake inhibitor, presenting weak but relatively selective inhibition characteristics of DA reuptake. Its potency as an inhibitor of NE reuptake is one-half of that of DA, and it shows little affinity for the serotonergic transport system (1). Piacentini et al. (26) have reported an increase in hippocampal NE and DA concentrations after Bup injection and a significant decrease in peripheral prolactin concentrations using brain microdialysis and catheterization methods. These results suggest that Bup influenced neurotransmission both at the central and at the peripheral level. However, they did not address how Bup influences thermoregulatory functions. While Bup has been reported to possess thermogenic properties (15, 16) possibly relating to its amphetamine-like structure (20), no studies have examined the hypothalamic catecholamine levels, brain temperature (Tbrain) and Tcore after Bup injection.

Therefore, the purpose of the present study was to investigate the effects of an acute dose of Bup on thermoregulatory responses and the extracellular NE, DA, and serotonin (5-HT) concentrations via in vivo microdialysis and biotelemetry methods in freely moving rats with special focus on the PO/AH, which is the most important control center of thermoregulation. Because of the well-documented effects of 5-HT on the regulation of a number of physiological functions and behaviors such as sleep, fatigue, pain, arousal, and thermoregulation (11, 14), we have also investigated 5-HT concentrations in the PO/AH.

MATERIALS AND METHODS

Animal treatment. Wistar male rats (300–350 g) were used in all experiments. Animals were housed in a room of normal ambient temperature, on a 12:12-h light-dark cycle (lights on at 0800). Animals had a standard diet with free access to food and water throughout the experiments. The procedures used in this study were carried out

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According to the European Guidelines on Animal Experimentation and were approved by the Ethic Committee of the Faculty of Medicine and Pharmacy of the Vrije Universiteit of Brussel.

Surgery. Animals were anesthetized with pentobarbital sodium (60 mg/kg ip). A telemetry device (model TA10TA-F20, Data Sciences International, St. Paul, MN) was implanted in the peritoneal cavity (9). After a 6-day recovery period, rats were anesthetized with pentobarbital sodium (60 mg/kg ip) and placed on a stereotaxic frame. The skull was exposed, and two intracerebral guides (model MAB 6.14.1C, Microbiotech, Stockholm, Sweden) were implanted. One guide was inserted in the left PO/AH (anterior: −0.3 mm, lateral: −0.8 mm, ventral: +6.7 mm, relative to bregma) for the microdialysis probe, and one in the right frontal cortex (anterior: +2.5 mm, lateral: +3.2 mm, ventral: +2.0 mm, relative to bregma) for the thermocouple probe to measure brain temperature (23). The cannula was secured to the skull using dental cement (Durelon, Espe, Germany). Postoperative analgesia was provided to each rat by giving a single injection of ketofen (4 mg/kg ip). Microdialysis experiments were carried out 48 h after implantation of the probes.

Experimental procedures. On the day of experiment, rats were anesthetized with 4% sevoflurane and oxygen insufflated into a transparent chamber. After induction, the rat was maintained under anesthesia to change the probes, using 1.5% sevoflurane delivered with oxygen at 0.8 l/min via a facemask (35). The guides were replaced by a microdialysis probe with a membrane length of 2 mm (model MAB 6.14.2, Microbiotech, Stockholm, Sweden) and a thermocouple probe in the prefrontal cortex (HYP-O-SLE, Omega, Stanford, CT). The thermocouple for tail skin temperature (Ttail) was also attached with tape on the dorsal surface of the skin ~10 mm from the base of the tail.

The microdialysis probes were connected to a microinfusion pump (model CMA 100, CMA Microdialysis, Stockholm, Sweden) and were perfused with Ringer solution (147 mM NaCl, 4 mM KCl, and 2.3 mM CaCl2) at a flow rate of 1 μl/min. Microdialysis sampling was started after 2 h of probe implantation. Then, after 2 h of baseline collections, rats randomly received either an intraperitoneal injection of 17 mg/kg of Bup hydrochloride, dissolved in physiological saline (GlaxoSmithKline, Hertfordshire, UK) (n = 6) or saline (1 ml/kg) (n = 6). This particular dosage of Bup was chosen from literature (21, 34) and our laboratory’s previous observation that this relatively low dose is sufficient to increase central neurotransmitter release (26).

Sampling. During the experiments, microdialysis samples (20 μl) were collected every 20 min for 180 min. The samples were protected from oxidation by addition of 5 μl of antioxidant solution: L-cysteine (3.3 mM), Na2EDTA (0.27 mM), acetic acid (100 mM), ascorbic acid (0.0125 mM). Tbrain, Tcore, and Ttail, an index of heat loss response (10, 13), were measured every 20 min.

Chromatographic assays for the determination of NE, DA and 5-HT in dialysates from PO/AH. For the analysis of NE, DA, and 5-HT, an offline microbore liquid chromatography assay was used with automatic injection (10 μl) of the samples, as described previously in detail (5, 31). In summary, the assay was based on ion-pair reverse-phase chromatography (C8, 5 μm; 100 × 1 mm), coupled to single-channel electrochemical detection (Decade, Leiden, The Netherlands). The mobile phase consisted of 27 ml acetonitrile and 200 ml of the following aqueous buffer: sodium acetate trihydrate (0.1 M), citric acid monohydrate (20 mM), decane sulfonic acid (2 mM), and sodium EDTA (0.5 mM) adjusted to pH 5.5. The flow rate through the column was 90 μl/min. Because of the high pH 5.5 of the mobile phase, a low oxidation potential was set (450 mV vs. Ag-AgCl). The retention times for NE, DA, and 5-HT were 3, 6, and 12 min, respectively with quantification limit for all compounds between 30 and 60 PM.

Histological examination. At the end of each experiment, rats were killed with an overdose of pentobarbital sodium, and the brain was removed. The position of the microdialysis probe was verified in coronal sections (100 μm thick) stained with Chinese ink according to the coordinates described by Paxinos and Watson (23). We confirmed the location of the tip of microdialysis probe in the PO/AH. A photomicrograph of a sample section is shown in Fig. 1A, where the tip of location (arrow) and the width of the probe can be seen. Figure 1B is a diagrammatic representation of the tip locations in 12 experiments, where the tips are correctly positioned into the PO/AH.

Data collection and statistical analysis. The average concentration of three microdialysis samples and temperature values for 1 h before drug administration was considered as the baseline and was defined as 100% or 0°C, respectively. Microdialysis samples and temperature values were expressed relative to this baseline value (means ± SE).
Differences between data were evaluated for statistical significance by using two-factor (time and conditions) repeated-measures ANOVA followed by Bonferroni/Dunn post hoc tests. \( P < 0.05 \) was regarded as statistically significant.

**RESULTS**

Figure 2 shows the mean changes in \( T_{\text{brain}} \) (A), \( T_{\text{core}} \) (B), and \( T_{\text{tail}} \) (C) before and after injection of Bup expressed at 20 min intervals. Injection of Bup influenced both \( T_{\text{brain}} \) and \( T_{\text{core}} \), with values significantly elevated compared with the baseline value (\( T_{\text{brain}}: 0.7 \pm 0.1^\circ \text{C}, T_{\text{core}}: 0.5 \pm 0.1^\circ \text{C}, 40 \) min after injection; \( P < 0.05 \)). These higher values of \( T_{\text{brain}} \) and \( T_{\text{core}} \) of Bup condition were significantly higher when compared with those recorded after saline treatment, and they were maintained until 100 and 120 min postinjection, respectively. \( T_{\text{tail}} \) was immediately decreased by the Bup injection, and a significant difference was found at 20 and 40 min (\(-1.9 \pm 0.8^\circ \text{C}, 40 \) min after injection; \( P < 0.05 \)) compared with the baseline levels and the corresponding time of saline treatment. Saline injection did not change thermal parameters (\( n = 6 \)).

Extracellular NE levels in the PO/AH increased significantly from 20 min after Bup injection compared with the baseline levels, reaching their highest value 40 min postinjection (466 ± 116%, Fig. 3A). These NE concentrations remained significantly elevated throughout the experiments (\( P < 0.05 \)), and NE values were significantly higher at 20, 40, 60, and 80 min postinjection when compared with those recorded after saline treatment. Extracellular DA levels in the PO/AH increased significantly from 40 min after Bup injection (Fig. 3B). They remained significantly elevated until 100 min postinjection compared with the baseline levels (\( P < 0.05 \)), and DA values were significantly higher at 40 and 60 min postinjection when compared with those recorded after saline treatment. The
peak value of DA was observed 40 min postinjection (235 ±
45%, Fig. 3B). 5-HT showed no significant change after Bup
injection (Fig. 3C). No such effect on neurotransmitters in the
PO/AH was apparent under saline injection. Baseline levels of
NE, DA, and 5-HT were 0.21 ± 0.1, 0.20 ± 0.1, and 0.25 ±
0.1 nM, respectively (n = 12).

DISCUSSION

The main finding of the present study was that an acute
injection of Bup induced an increase in Tbrain and Tcore with a
decrease in heat loss responses. These thermal responses were
accompanied by an increase of the extracellular concentrations
of NE and DA with no effect on 5-HT release in the PO/AH of
freely moving rats measured via in vivo microdialysis.

Bup is a weak monoamine reuptake inhibitor that shows
2.5-fold selectivity for DA vs. NE and that has no effect on 5-HT
(12, 30). Previous microdialysis studies have shown that
acute doses of Bup have an effect on DA release in the rat
striatum and nucleus accumbens in a dose-dependent manner
(21) and on hippocampal NE and DA release of rats (26). It
was also shown in mice that Bup (30 mg/kg ip) increased NE
and DA release in the frontal cortex (40). In the present study,
an acute injection of Bup increased the extracellular concen-
trations of NE and DA as well, but not of 5-HT, in the PO/AH
(Fig. 3), which is comparable to the effect in the frontal cortex
in mice (40). The NE increase was faster in onset (20 min) than
the DA increase in the PO/AH, and NE releases were higher
than those of DA. These results suggest that Bup influences
more the noradrenergic neural activity than the dopaminergic
neural activity in the hypothalamus.

The effects of Bup on thermoregulation have been previ-
ously reported in mice (39) and in rats (15, 16). Consistent with
our observations, Liu et al. (15) demonstrated that oral admin-
istration of Bup increases colonic temperature in rats, with a
rapid rise in oxygen consumption at a higher dose (30 mg/kg).
These results imply that Bup has thermogenic properties in
rats. However, there is no study that examined the effect of
Bup on acute thermoregulatory responses and hypothalamic
catecholamine levels in freely moving animals. In the present
study, the acute injection of Bup increased NE and DA but not
5-HT in the PO/AH in rats, which caused heat production and
thus increased Tbrain and Tcore. The decreased Ttail indicates a
suppression of heat loss mechanisms, probably because of a
vasoconstriction (8).

It is well known that NE, DA, and 5-HT in the hypothalamic
regions play essential roles in several homeostatic functions,
including thermoregulation (7, 36). For example, local appli-
cation of NE into the rat PO/AH causes an increase in Tcore (4),
and NE inhibits the activities of warm-sensitive neurons in the
PO/AH (36). These findings suggest that NE is involved in heat
production mechanisms. On the other hand, Quan et al. (28, 29)
have shown that NE microdialyzed into the preoptic area of
conscious guinea pigs evokes a fall in Tcore that is mediated by
a reduction in metabolic rate. It has also been reported that DA
excites the firing rate of warm-sensitive neurons but inhibits
cold-sensitive neurons in tissue slices of the PO/AH (32). In
addition, microinjections of DA or apomorphine, a DA agonist,
into the hypothalamus and substantia nigra of the rat produced
a DA-mediated decrease in temperature (3, 7). Moreover, DA
release in the PO/AH is enhanced during treadmill exercise (9)
and DA induces an increase in Tcore in rats (19). Thus the
specific role that these monoamines play in the hypothalamus
remains unclear. However, the present results suggest that both
NE and DA or one of the two might be involved not only in
thermogenesis but also in heat dissipation because Ttail imme-
diately dropped after Bup injection.

Several studies have examined the relationship between
5-HT and thermoregulation as well. Local application of 5-HT
into the PO/AH was reported to alter the activities of thermo-
sensitive neurons (36). In the present study, although Tbrain,
Tcore, and Ttail were influenced by acute injection of Bup, the
extracellular 5-HT in the PO/AH remained unaltered. In addi-
tion, our laboratory’s recent study showed that the perfusion of
a 5-HT reuptake inhibitor or 5-HT1A agonist, into the PO/AH
did not affect Tcore despite the fact that extracellular 5-HT in
the PO/AH was increased or decreased (13). Therefore, hypo-
thalamic 5-HT does not seem to mediate thermoregulation in
response to immediate fluctuations in temperature, and 5-HT in
the PO/AH is not involved in the Bup-induced increase in Tcore.

Different reuptake inhibitors in humans have been used to
evaluate the effects of an increased neurotransmission on
exercise performance and on CNS fatigue (17, 18, 22, 24, 25,
27, 33, 38). Recently, we found that acute ingestion of Bup
improved time trial exercise performance of cyclists in a warm
environment (30°C) (37). However, this increased performance
was accompanied by a rise in rectal temperature during
exercise reaching 40°C or above. Noteworthy, this response
appeared to occur without any change in the subjective sensa-
tion. These results suggest that during exercise in the heat, Bup
may override the inhibitory signals arising from the CNS that
induce to stop exercising when close to the critical tempera-
ture. The pharmacological profile of Bup is different in animals
and humans due to the fact that rodents lack hydroxybupro-
pion, the major metabolite of Bup (6). However, if the results
of the present study can be transposed to humans, they suggest
that Bup probably also increases human brain temperature.

Conclusion. The data from this study suggest that acute
injection of Bup acts on the brain and affects brain and core
temperatures through an increase in NE and DA release in the
PO/AH. Further studies are necessary to elucidate the exact
role of both neurotransmitter systems in heat production and
heat loss mechanisms.

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REFERENCES

1. Ascher JA, Cole JO, Colin JN, Feighner JP, Ferris RM, Fibiger HC,
Golden RN, Martin P, Potter WZ, and Richelson E. Bupropion: a
review of its mechanisms and antidepressant activity. J Clin Psychiatry
2. Boulant JA and Dean JB. Temperature receptors in the central nervous
3. Brown SJ, Gisolfi CV, and Mora F. Temperature regulation and dopa-
minergic systems in the brain: does the substantia nigra play a role? Brain

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