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# DOPAMINE $D_2$ RECEPTOR STIMULATION INHIBITS COLD-INITIATED THERMOGENESIS IN BROWN ADIPOSE TISSUE IN CONSCIOUS RATS

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**Abstract—Dopamine $D_2$-like receptor agonists cause hypothermia. We investigated whether inhibiting heat production by interscapular brown adipose tissue (iBAT), a major thermogenic organ in rats, contributes to hypothermia caused by dopamine $D_2$-like receptor agonists. Temperature of iBAT and tail artery blood flow were measured in conscious rats. Activity in postganglionic sympathetic nerves supplying iBAT was assessed in anesthetized rats. Conscious rats were housed in a warm cage maintained at 26–28 °C and then transferred to a cold cage at 5–10 °C to induce iBAT thermogenesis. Cold exposure increased iBAT temperature (+0.7±0.1 °C, 30 min after transferring to the cold cage, $P<0.01$, $n=54$). The mixed dopamine $D_2/D_3$ receptor agonist, 7-hydroxy-2-(di-n-propylamino)tetralin (7-OH-DPAT, 0.5 mg/kg s.c.) reversed the cold-induced increase in iBAT temperature (−2.8±0.2 °C at 30 min after 7-OH-DPAT treatment during cold exposure vs. +0.3±0.1 °C at 30 min after vehicle treatment during cold exposure, $n=8$). These temperature changes were blocked by pre-treatment with the $D_2$ receptor antagonists spiperone (20 μg/kg i.p.) and L-741,626 (2.5 mg/kg i.p.), but not by the selective $D_3$ receptor antagonist SB-277011A (10 mg/kg i.p.). Another mixed dopamine $D_2/D_3$ receptor agonist, quinpirole (0.5 mg/kg s.c.) also reversed cold-induced iBAT thermogenesis, and this effect was also prevented by pre-treatment with spiperone, but not with a peripherally acting dopamine receptor antagonist, domperidone (2 mg/kg s.c.). Neither 7-OH-DPAT nor quinpirole reversed cutaneous vasoconstriction elicited by cold exposure. In anesthetized rats, quinpirole (0.5 mg/kg i.v.) abolished iBAT sympathetic nerve discharge elicited by cooling the trunk, and this change was reversed by spiperone (20 μg/kg i.v.). These results demonstrate that activation of CNS dopamine $D_2$ receptors inhibits sympathetically-mediated iBAT thermogenesis in response to cold exposure. Furthermore, they suggest that in rats hypothermia induced by dopamine $D_2$ receptor agonists in cold environments is mainly due to decreased heat production rather than to increased heat loss.** © 2007 IBRO. Published by Elsevier Ltd. All rights reserved.

**Key words: iBAT, body temperature regulation, Parkinson's disease, sympathetic nerve activity, quinpirole, 7-OH-DPAT.**

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*Abbreviations:* iBAT, interscapular brown adipose tissues; SCVARs, sympathetic cutaneous vasomotor alerting responses; 7-OH-DPAT, 7-hydroxy-2-(di-n-propylamino)tetralin.

Evidence suggests that the hypothermic effect of apomorphine reflects stimulation of dopamine $D_2$-preferring receptors in the CNS (Cox et al., 1978, 1981; Cox and Lee, 1980; Clark and Lipton, 1985; Carboni et al., 1986; Faunt and Crocker, 1987; Chipkin, 1988; Ogren and Fuxe, 1988). Since both heat production by the body and heat exchange between the body and the environment contribute to body temperature, the hypothermic effect of dopamine $D_2$-preferring agonists could be due to a decrease in heat production or an increase in heat loss or a combination of both factors. An increase in thermoregulatory cutaneous blood flow, leading to enhanced heat loss, contributes to apomorphine-induced hypothermia (Cox et al., 1978). In addition apomorphine-induced hypothermia is associated with decreased oxygen consumption, indicative of a decreased metabolic rate (Cox et al., 1981). In rats, brown adipose tissue, including interscapular brown adipose tissue (iBAT), is a major site of sympathetically-controlled thermogenic metabolism. Increased activation of iBAT, with heat production, is part of the natural facultative thermogenic response to cold exposure (Morrison, 2004b). To date the effect of dopamine $D_2$-preferring receptor agonists on the sympathetic control of iBAT thermogenesis has not been investigated. Pilot experiments (Ootsuka and Blessing, unpublished observations) demonstrated that systemically administered apomorphine inhibits iBAT thermogenesis. In the present study, we investigated the effect of quinpirole (LY171555) and 7-OH-DPAT (7-hydroxy-2-(di-n-propylamino)tetralin), mixed dopamine $D_2/D_3$ receptor agonists (Sokoloff et al., 1990; Sautel et al., 1995; Levant, 1997; Heidbreder and Hagan, 2005), on iBAT thermogenesis, because they have been shown to reduce body temperature (Faunt and Crocker, 1987; Kurashima et al., 1995).

Using conscious rats with chronically implanted probes to measure iBAT temperature and to measure tail artery blood flow, we examined whether quinpirole and 7-OH-DPAT inhibit cold-induced iBAT thermogenesis and cutaneous vasoconstriction. We determined whether pre-treatment with the dopamine $D_2$ receptor antagonist spiperone (Sokoloff et al., 1990; Levant, 1997) prevented the action of quinpirole and whether spiperone, L-741,626 (another dopamine $D_2$ receptor antagonist) (Kulagowski et al., 1996), the peripherally-acting $D_2$ receptor antagonist domperidone and SB-277001A, a selective dopamine $D_3$ receptor antagonist (Reavill et al., 2000) prevented the action of 7-OH-DPAT on cold-induced iBAT thermogenesis and cutaneous vasoconstriction. We also directly measured iBAT sympathetic nerve activity in anesthetized rats and determined whether quinpirole inhibits the increase in iBAT sympathetic nerve activity induced by cold exposure.

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## EXPERIMENTAL PROCEDURES

Experiments were conducted in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC), and were approved by the Flinders University Animal Welfare Committee. All efforts were made to minimize the number of animals used and their suffering.

### Surgical preparation in conscious rats

Male Sprague–Dawley rats (250–325 g) were anesthetized with isoflurane (Veterinary Companies of Australia Pty. Ltd., King's Park, NSW, Australia). A Doppler ultrasonic flow probe (Iowa Doppler Products, Iowa City, IA, USA) was implanted around the tail artery about 3 cm from the base. Probe wires were passed s.c. and attached to a head socket (Garcia et al., 2001). The tip of a telemetry temperature probe (TA-F40 W/TP, Data Sciences International, Transoma Medical, St. Paul, MN, USA) was positioned in iBAT and the transmitter body of the probe was placed in the peritoneal cavity (Ootsuka and Blessing, 2006). A Subcue temperature probe (Subcue Dataloggers, Calgary, AB, Canada) was also placed in the peritoneal cavity (Ootsuka and Blessing, 2006). Insulated wires from the two-pin connector on the Subcue probe were attached to pins on the head socket, so that the probe could be reset after downloading the data in each experiment, without removing the probe from the animal. Animals recovered from anesthesia and were returned to the animal house for at least 1 week before experimental studies. Each rat used in the study had a correctly positioned iBAT probe, as demonstrated by selecting only those animals in which iBAT temperature increased by at least 0.5 °C when the animal was placed in a cold (5–10 °C) environment for 30 min.

### Experimental design in conscious rats

On the day of the experiment, between 10:00 a.m. and 2:00 p.m., during the animals' light cycle, a rat was transferred from the animal house to our experimental room. The rat was placed in a cage fitted with a swivel device and a flexible cable that could be plugged into the animal's head socket. A receiver for the telemetric probe was placed under the cage. Cold exposure was performed by transferring animals manually from a warm cage (25–28 °C) to a cold cage (5–10 °C). Animals were also held manually during s.c. injection of drugs.

There were eight experimental groups. Rats were first kept in the warm cage in all groups (Groups A to H).

In four of the eight groups (Groups A, C, F and G), parameters were continuously recorded for 60 min, followed by the first administration of either vehicle (0.5 ml, i.p., Groups A and C), L-741,626 (2.5 mg/kg, i.p., Group F) or SB-277011A (10 mg/kg i.p., Group G). The rat was left undisturbed for 60 min, and then transferred from the warm cage to the cold cage. After 30 min, the cage's door was opened and animals were treated with either 7-OH-DPAT (0.5 mg/kg, s.c., Groups A, C and G) or vehicle (0.5 ml, i.p., Group F). The rat was again left undisturbed for 30 min in the cold cage and then returned to the warm cage.

In the remaining four of the groups (Groups B, D, E and H), the timing of transfer to the cold cage after the first administration of drugs was different from that described for the previous four groups. The rat was left undisturbed for 10 min (instead of 60 min) after the first administration of either vehicle (0.5 ml, i.p., Group B), spiperone (0.02 mg/kg, i.p., Groups D and E), or domperidone (2.0 mg/kg s.c. Group H). Then the rat was transferred from the warm cage to the cold cage. After 30 min, the cage's door was opened and a second injection of either quinpirole (0.25 mg/kg s.c., Groups B, D and H) or 7-OH-DPAT (0.5 mg/kg s.c., Group E) was made. The rat was again left undisturbed for 30 min in the cold cage before being returned to the warm cage.

### Surgical preparation in anesthetized rats

Male Sprague–Dawley rats (390–446 g) were anesthetized with isoflurane. After shaving the trunk and limbs, an endotracheal tube was inserted via a tracheotomy. The right femoral artery and vein were cannulated for measurement of systemic arterial pressure and i.v. drug administration, respectively. Isoflurane anesthesia was then replaced with a cocktail of urethane (400–800 mg/kg i.v.) and $\alpha$-chloralose (40–80 mg/kg i.v.) (50 mg of $\alpha$-chloralose dissolved in 10 ml of 10% 2-hydroxypropyl-$\beta$-cyclodextrin; Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) (Storer et al., 1997). The level of anesthesia was maintained at a depth sufficient to abolish withdrawal reflex. iBAT sympathetic nerve was dissected as described in Ootsuka and McAllen (2006). Nerve signals were recorded (NL100 pre-amplifier, Digitimer, Welwyn Garden City, Hertfordshire, UK), amplified (gain 20,000, NL104 amplifier, Digitimer) and filtered (1–200 Hz, NL125 filter, Digitimer). Rats were paralyzed with pancuronium bromide (1 mg/kg i.v.) and then ventilated artificially with 100% $O_2$. End expiratory $CO_2$ concentration was monitored continuously with a $CO_2$ monitor (Normocap, Datex, Helsinki, Finland), and was maintained between 3.5% and 4.5% (resting condition) by adjusting ventilation volume. The animal was allowed to recover from paralysis between doses so that adequate anesthesia could be confirmed before paralysis was re-established. The temperature of the contralateral iBAT, abdominal skin and colon was measured with thermocouples. The skin of the rat's thorax and abdomen was then cooled by perfusing ice cold water (5–10 °C, for 1–6 min) through a water jacket, and the consequent increase in iBAT sympathetic nerve discharge was used to confirm that recording was from nerves supplying the BAT. At the end of the experiment, ganglionic blockade with chlorisondamine chloride (10 mg/kg i.v.) was administered to confirm loss of nerve activity and thus nerve recording from sympathetic axons.

### Drugs

All drugs were freshly prepared and injected s.c. in a volume of 0.5 ml.

Spiperone, (−)-quinpirole HCl (quinpirole), 7-OH-DPAT and L-741,626 (Sigma, Castle Hill, NSW, Australia) were dissolved in Ringer. Domperidone (Sigma) was dissolved in acidified Ringer. SB-277011A (kindly provided by GlaxoSmithKline, Verona, Italy) was dissolved in 10% hydroxypropyl-$\beta$-cyclodextrin. Drug doses were determined in pilot studies and from previously published studies.

### Data recording and analysis

Blood flow signals from Doppler ultrasonic flow probes and temperature signals from DSI telemetry probes were recorded with MacLab and Chart (ADInstruments, Castle Hill, NSW, Australia). Flow was digitized at 40 Hz and BAT temperature was digitized at 2 Hz. Temperature data from the Subcue probe were stored in its memory every 1 min during experiments and were downloaded after each experiment. Data were analyzed with Chart, Igor Pro (WaveMetrics, Lake Oswega, OR, USA) and Statview (SAS Institute, Cary, NC, USA) software. Details of measurement times are given in the appropriate Results section.

In anesthetized rats, all data were digitized with MacLab and captured into a computer with Chart. iBAT sympathetic nerve activity was recorded with bipolar silver electrodes (band pass filter 1–1000 Hz, gain 20,000), and digitized at 400 Hz after passing through the 200 Hz built-in low pass filter of MacLab. iBAT, skin and rectal (body) temperatures were digitized at 10 Hz. The amplitude of iBAT sympathetic nerve activity was obtained by calculating total power spectral density between 0 and 20 Hz from the autospectra of sequential 5.12-s segments of iBAT sympathetic nerve activity.