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Effect of Conventional (Mixed β_1/β_2) and Novel (β_3) Adrenergic Agonists on Thermoregulatory Behavior

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CARLISLE, H. J. AND M. J. STOCK. *Effect of conventional (mixed β_1/β_2) and novel (β_3) adrenergic agonists on thermoregulatory behavior.* PHARMACOL BIOCHEM BEHAV **40**(2) 249–254, 1991.—The effects of submaximal and maximal thermogenic doses of isoproterenol (ISO) on operant thermoregulatory responses in a cold (−8°C) environment were tested in lean (+/?) Zucker rats trained to barpress for radiant heat. Contrary to expectations, ISO rats pressed for twice as much exogenous heat as controls, but showed a smaller rise in colonic temperature. Conversely, a β_3-selective adrenergic agonist (RO40-2148) decreased the requirement for exogenous heat and produced larger rises in colonic temperature. RO40-2148 and another β_3-agonist (ICI D7114) produced similar responses in obese (fa/fa) Zucker rats, but tests with ISO were terminated because it caused profound, and lethal hypothermia. The hypothermic effects of ISO on colonic temperature were also observed in Sprague-Dawley rats at room temperature (22°C), whereas RO40-2148 produced hyperthermia. These results provide behavioral evidence for the high thermogenic selectivity of these novel adrenergic agonists and support the existence of an atypical β_3-adrenoceptor. The hypothermic effects of ISO are presumed to be due to actions on β_1- and/or β_2-adrenoceptors.

Isoproterenol RO40-2148 ICI D7114 metabolite Body temperature Operant responses Thermoregulatory behavior

THE sympathetic nervous system is known to play a major role in the activation of adaptive (or facultive) forms of thermogenesis, such as nonshivering and diet-induced thermogenesis, with brown adipose tissue (BAT) being the major effector organ [see (8,29) for reviews]. Exogenous norepinephrine mimics the effect of sympathetic stimulation and raises heat production in a dose-dependent manner by up to 50–200% in small rodents. The increase in heat production depends on the animal's adaptive response to a variety of environmental thermal and dietary influences, and has been widely used to assess changes in thermogenic capacity [for examples, see (12, 18, 21)]. Studies utilising selective α- and β-adrenergic antagonists have shown that stimulation of thermogenesis by the sympathetic nervous system or by exogenous norepinephrine is almost entirely due to activation of β-adrenoceptors (24,25), with perhaps only a small α-adrenoceptor component (4, 10, 28). Likewise, BAT thermogenesis in vitro is mediated by β-adrenoceptors, and β-adrenergic agonists such as isoproterenol are often used instead of norepinephrine for in vivo and in vitro studies of thermogenesis.

In recent years, a group of novel β-adrenergic agonists (e.g., BRL35135, RO40-2148, ICI D7114) have become available for pharmacological and other investigations of thermogenesis. The common feature of these agonists is that they have all been developed as potential thermogenic drugs for the treatment of obesity, and for this reason have been designed to be relatively free of the β_1- and β_2-mediated effects (increased heart rate, blood pressure, bronchodilation, tremor, etc.) produced by conventional agonists. These novel compounds have proved to be highly selective for thermogenesis, and are 20–400 times more potent as agonists of BAT thermogenesis than as agonists of atrial rate (β_1-effect) or tracheal relaxation (β_2-effect) (1, 2, 15). The high BAT-selectivity of these agonists, and the low inhibitory activity of conventional β-adrenergic antagonists on thermogenesis led to the notion that the BAT β-adrenoceptor was atypical (1). A gene sequence corresponding to a third β-receptor (β_3) has now been identified and cloned (7), and it is generally assumed that this is the atypical BAT adrenoceptor and that the novel BAT-selective thermogenic drugs are β_3-agonists, although there has been some debate about this (9,30).

Given the potent effects of β-adrenergic agonists on heat production, it is not surprising to find that body temperature increases, particularly in warm or thermoneutral environments (20–30°C). In their collation of body temperature responses to various drugs, Clark and Lipton (5) list numerous reports of increases in temperature of up to 4°C following peripheral administration of norepinephrine or isoproterenol, the increase depending on dose, ambient temperature and prior treatment (e.g., cold acclimation). However, there have been occasional

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observations of decreases in body temperature (6, 11, 13, 17), and in a more recent study norepinephrine was found to decrease core temperature and increase operant responses for heat in rats exposed to −8°C (31).

The idea that potent thermogenic agonists can cause hypothermia runs counter to expectations, but raises the possibility that in certain situations these paradoxical effects predominate due to actions on different receptor subtypes at different sites from those responsible for thermogenesis. In addition, the ambient temperature and the animal's ability to indulge in thermoregulatory behavior have to be taken into account, since the majority of studies reporting hyperthermic responses have been carried out at, or close to thermoneutrality and/or involve some form of restraint or confinement. Given these considerations and the recent availability of several β_3-agonists, it was decided to investigate behavioral thermoregulatory responses to a conventional, mixed (β_1/β_2) adrenoceptor agonist (isoproterenol) and one of the novel β_3-selective agonists (RO40-2148). A preliminary summary of this work has been reported (27).

METHOD

Animals

For the operant thermoregulatory tests, lean (+/?) and obese (fa/fa) Zucker rats were bred at University of California, Santa Barbara from stock derived from the colony maintained at University of California, Davis. The animals were 8-month-old males and females, and free from mycoplasmosis. The animals were fed a standard stock diet (Purina 5001) ad lib and housed in groups of 2–4 in a room maintained at 22°C with 50% RH and a 12:12 light:dark cycle (lights on at 0700 h); all testing was carried out during the light phase of the cycle. For thermoregulatory tests at normal (22°C) room temperature, 3-month-old male Sprague-Dawley rats purchased from Bantin & Kingman (Fremont, CA) were housed 2 per cage under identical conditions, but no other animals were housed in the room and on experimental days access was restricted to the researchers.

Body Temperature

The animal was removed from the home or experimental cage and colonic temperature taken within 2 min using a Sensortek (Clifton, NJ) BAT-12 meter and thermocouple probe inserted via the rectum at least 6 cm, or until peak temperature was indicated. All temperature recordings were made with the animal sitting unrestrained on a bench.

Barpress Apparatus

The apparatus consisted of a circular wire-mesh cage of 22 cm diameter and 22 cm depth with Plexiglas-rod flooring. A 3 × 4 cm Plexiglas lever mounted on a microswitch (7 g activation mass) protruded 5 cm into the cage 2 cm above the floor. Two 250-W red-bulb infrared lamps were mounted each side of the cage at a 45° angle to the floor, and focused on the rat when at the lever. The irradiance of the lamps was measured with an Eppley thermopile placed in the position normally occupied by the rat at the lever, and the total power dissipated by the lamps set at 300 W to provide 180 mW/cm^2 irradiance [for further details see (22)]. The apparatus was placed in a 0.48 m^3 freezer maintained at −8 ± 2°C. Low-level background lighting was provided by a red 25-W incandescent lamp mounted outside the cage. The heat lamps remained activated as long as the lever was depressed, and equipment in an adjoining room provided a cumulative record of the pattern of responding as well as the number of barpresses and duration (s) of heat lamp activation. For some experiments, the duration of heat lamp activation was printed-out at 10-min intervals.

Barpress Procedure

The trunk of the animal was shaved closely (Oster clipper, No. 40 blade) the day prior to each test. All animals acquired the barpress response during an initial 2–4-h training test, and were given at least five additional tests at 3–4-day intervals to ensure stable performance before starting any experiments. The test procedure involved recording colonic temperature on removing the animal from its home cage, measuring body weight and injecting the appropriate drug or vehicle before placing the animal in the apparatus for 90 min. Barpress responses and duration of heat reinforcements were noted at 30-min intervals.

Drugs

(−)-Isoproterenol HCl (ISO) was obtained from Sigma (St. Louis, MO), RO40-2148 (RO40) from Hoffmann-La Roche (Basel, Switzerland) and ICI D7114 acid metabolite (ICI) from ICI Pharmaceuticals (Macclesfield, UK). RO40 is very lipophilic and had to be prepared for injection as a suspension by dissolving in a minimum volume of dimethylsulphoxide (DMSO; Sigma), making up to volume with 5% gum arabic in normal saline (0.9% NaCl) and sonicating before use. The other drugs were prepared in the same way, and the control vehicle was 10% DMSO in 5% gum arabic/saline. All injections (1 ml/kg) were subcutaneous. The doses of ISO, RO40 and ICI used were selected on the basis of dose:responses for oxygen consumption measured previously in Sprague-Dawley rats at thermoneutrality using closed-circuit chambers (26) at St. George's Hospital Medical School (London).

Experimental Protocols

Experiment 1 involved measuring operant responses at −8°C (as described above) of six weight-matched, lean female Zucker rats to submaximal (approximately ED_{50}) thermogenic doses of ISO (30 μg/kg) and RO40 (500 μg/kg). Experiment 2 investigated the effects of maximal thermogenic doses of ISO (75 μg/kg) and RO40 (5 mg/kg) on colonic temperature in eight male Sprague-Dawley rats in their home cage at 22°C. Measurements were made prior to injections, and every 30 min thereafter for 2.5 h. Experiment 3 was a repeat of Experiment 1 (operant responses at −8°C) using groups of four male and four female lean Zucker rats (matched for weight) injected with the higher (maximal) doses of ISO and RO40. Experiment 4 compared the operant responses at −8°C of groups of four male and four female obese Zucker rats injected with maximal thermogenic doses of RO40 (5 mg/kg) and ICI (1.4 mg/kg).

Data Analysis

The colonic temperature responses of rats studied at 22°C were assessed from the 30-min changes relative to the preinjection value. The barpress experiments were more difficult to analyse because of the problem of trying to compare simultaneously changes in heat influx (mW/cm^2) with changes in colonic temperature (°C). This was overcome by transforming the data to common units of thermal balance (kJ), calculated as either Net Thermal Balance [NTB, kJ = Heat Influx (HI) − Change in heat stored (dS)], or as Net Thermal Efficiency (NTE% = dS/HI × 100). Heat influx was calculated using Equation 9 of

TABLE 1

EFFECT OF SUBMAXIMAL THERMOGENIC DOSES OF ISOPROTERENOL AND RO40-2148 ON OPERANT RESPONDING FOR HEAT

	Tin (°C)	Tout (°C)	dT (°C)	BP/min (presses/min)	R/min (s/min)	R/BP (s/press)	dS (kJ)	HI (kj)	NTB (kJ)	NTE (%)
CON	37.47 ±0.40	38.53 ±0.33	1.07 ±0.38	2.60 ±0.24	17.10 ±1.26	6.85 ±0.79	1.02 ±0.36	27.28 ±1.98	26.26 ±2.04	3.87 ±1.34
ISO	37.28 ±0.37	37.95* ±0.23	0.67 ±0.34	2.18 ±0.20	25.97† ±2.14	12.49* ±1.61	0.67 ±0.33	42.19† ±3.16	41.52† ±3.22	1.67 ±0.70
RO40	36.67 ±0.23	38.67 ±0.33	2.00 ±0.18	2.61 ±0.21	13.70 ±1.25	5.55 ±0.85	2.00* ±0.20	24.16 ±2.46	22.16 ±2.43	8.62* ±1.10

Mean values ± SEM; n = 6 female, lean Zucker rats. *$p<0.05$, †$p<0.01$ vs. CON.

Explanation of column heads: Tin, Tout = Tcolonic before and after 90 min test; dT = change in Tcolonic; BP = barpresses; R = heat reinforcements; dS = change in body heat content; HI = heat influx; NTB = net thermal balance; NTE = net thermal efficiency.

Refinetti and Carlisle (22), and the change in heat storage calculated from the change in colonic temperature, body mass and the specific heat of the body (assumed to be 3.47 J/g). The rationale for these derived measures is that they are analogous to the energy balance terms used in studies of metabolic efficiency. Thus some of the energy derived from food intake is converted to body mass, and the remainder lost primarily as heat. Similarly, of the amount of radiant energy absorbed, some goes to increasing body heat content and the remainder is lost as heat.

Statistical Analysis

All results have been expressed as mean values ± SEM, and differences between vehicle (CON) and drug-treated group means assessed using Student's *t*-test for unmatched data; quoted probabilities are two-tailed.

RESULTS

Experiment 1

The mean changes in colonic temperature, barpress activity and derived values for thermal balance following submaximal doses of ISO and RO40 are shown in Table 1. It should be noted that the vehicle-treated group (CON) barpress for sufficient heat to raise colonic temperature by about 1°C. However, much of the heat influx (HI) is lost (NTB), and the amount retained (dS) accounts for only 4% (NTE) of the radiant heat supplied.

The colonic temperature of the ISO-treated animals was significantly lower than CON values at the end of the 90-min test, in spite of barpressing for more heat (heat reinforcements/min was increased by 50% compared to CON). The rate of barpressing was not affected, but the duration of each press was increased by over 80%. As a consequence, heat influx and net thermal balance were both increased by over 50%, but net thermal efficiency decreased to below 2%, although this decrease was not significant. RO40 had relatively small, but opposite effects to those of ISO. Compared to controls, RO40 rats showed a significantly greater increase in total body heat content (dS) due to a doubling in net thermal efficiency.

Experiment 2

The changes in colonic temperature following maximal thermogenic doses of ISO and RO40 in rats in their home cages at 22°C are shown in Fig. 1. The most striking feature seen is the marked decrease in colonic temperature of the rats receiving ISO. This decrease reached its nadir (−0.76°C) 90 min after injection, and colonic temperature was still less than the preinjection value, and significantly lower than CON values at the end of the 2.5-h test. Rats receiving vehicle (CON) exhibited a sustained and stable elevation in temperature (0.6–0.8°C) throughout the postinjection period, whereas those receiving RO40 showed a larger rise (0.8–1.3°C), that was significantly greater than that in CON rats over the first hour postinjection.

Experiment 3

The effects of maximal doses of ISO and RO40 on heat reinforcements are shown as 10-min records of barpress activity (secs heat/min) in Fig. 2, which reveals that throughout the 90-min test period the ISO-treated rats demand approximately twice as much heat as those injected with vehicle or RO40. The RO40-treated rats show a tendency to press for less heat than controls, but this was never statistically significant. Table 2

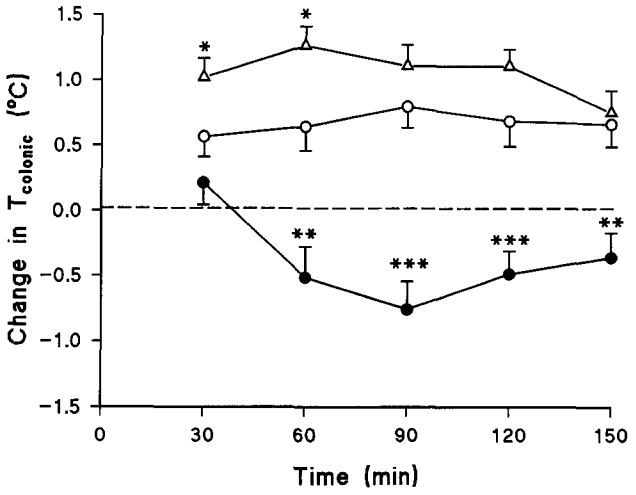

FIG. 1. Changes in colonic temperature in Sprague-Dawley rats relative to preinjection values following vehicle (open circles), 75 μg ISO/kg (closed circles) and 5 mg RO40/kg (triangles). Mean values with SEM (bar); n = 8. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to vehicle.

TABLE 2

EFFECT OF MAXIMAL THERMOGENIC DOSES OF ISOPROTERENOL AND RO40-2148 ON OPERANT RESPONDING FOR HEAT

	Tin (°C)	Tout (°C)	dT (°C)	BP/min (presses/min)	R/min (s/min)	R/BP (s/press)	dS (kJ)	HI (kJ)	NTB (kJ)	NTE (%)
CON	36.69 ±0.09	38.31 ±0.12	1.63 ±0.12	2.49 ±0.31	10.77 ±1.12	4.64 ±0.63	2.21 ±0.23	22.87 ±3.04	20.66 ±2.96	10.49 ±1.55
ISO	36.48 ±0.15	37.31† ±0.22	0.84* ±0.24	1.74 ±0.20	23.52‡ ±1.63	15.09‡ ±2.28	0.97 ±0.23	48.55‡ ±4.32	47.58‡ ±4.41	2.22‡ ±0.55
RO40	36.52 ±0.10	38.63 ±0.10	2.11* ±0.14	1.98 ±0.08	8.04* ±0.48	4.14 ±0.29	2.58 ±0.22	19.29 ±2.45	16.71 ±2.36	14.58 ±1.75

Mean values ± SEM; n = 8; 4 male plus 4 female, lean Zucker rats. *$p<0.05$, †$p<0.01$, ‡$p<0.001$ vs. CON.
See Table 1 for explanation of column heads.

summarises the data for the 90-min test and shows that the 2-fold increase in heat reinforcements in ISO rats was due to a 3-fold increase in barpress duration, with a slight, nonsignificant decrease in the rate of pressing (BP/min). Compared to CON rats, the rise in colonic temperature was 50% less in ISO rats, and heat influx and net thermal balance more than doubled; net thermal efficiency was reduced from 10% in CON to 2% in ISO rats. Unlike the submaximal dose of RO40 (Table 1), maximal doses produced significant, 30% greater increases in colonic temperature than the vehicle, and this was achieved with a 25% reduction in heat reinforcements. However, these changes were not sufficient to produce significant effects on the derived parameters of thermal balance. It should be noted that there were no qualitative differences in the drug responses between male and female rats in the treatment groups, which were balanced for sex and body weight.

Experiment 4

The effects of maximal thermogenic doses of the two β_3-adrenergic agonists, RO40 and ICI, on the thermoregulatory behavior of obese Zucker rats are summarised in Table 3. It should be noted that the behavior of the obese CON rats in these tests were very similar to that of lean CON rats (see Table 2), but the greater body mass of the obese rats results in higher values for heat storage, influx and net thermal balance. There was a tendency for RO40 rats to show greater rises in colonic temperature and reduced responses for heat compared to CON rats, but these changes were not statistically significant. However, the responses in ICI rats were significantly greater and were accompanied by a 45% increase in heat storage and a 65% increase in net thermal efficiency (Table 3).

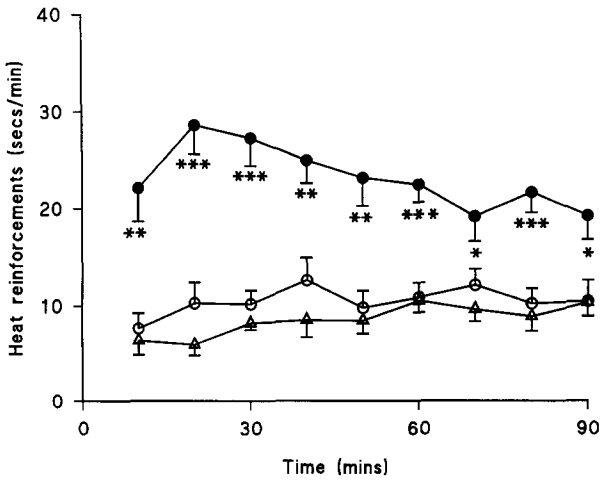

FIG. 2. Ten-min records of barpress activity (s of heat/min) of lean Zucker rats injected with vehicle (open circles), 75 μg ISO/kg (closed circles) or 5 mg RO40/kg (triangles) at time zero. Mean values with SEM (bar), n = 8. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to vehicle.

DISCUSSION

The operant response of rats exposed to −8°C has two components—the frequency and duration of barpresses. In these experiments frequency was maintained fairly constant and thermoregulatory responses were achieved by varying duration, i.e., seconds of heat per barpress. However, given a situation where the animal adjusts its thermoregulatory behavior as a "trade-off" between a preferred core temperature against a preferred skin temperature, it is possible for an intervention, such as injection with a thermogenic agonist, to produce statistically nonsignificant changes in the two measured parameters of thermoregulation (heat reinforcements and colonic temperature), but which together represent a significant change in thermal balance. In order to avoid overlooking such changes and assess the combined impact of simultaneous adjustments in core temperature and heat reinforcements, the parameters of thermal balance (dS, HI, NTB and NTE) were introduced into the analysis. Their use was best illustrated in Table 1, where the changes in colonic temperature and heat reinforcements of RO40 rats were not sufficiently large to reach statistical significance, but the changes in dS and NTE were. When it comes to ISO-treated rats, however, the thermal balance calculations merely serve to emphasize the obvious, but paradoxical effects of this thermogenic agonist on operant responses for heat.

Although the ED_{50} thermogenic doses of ISO and RO40 produced significant and opposite effects on operant responses (Experiment 1), it was decided to use higher doses for subsequent experiments in order to enhance the ISO effect. A supramaximal dose (100 μg/kg) was chosen initially, but this produced occasional fatalities, apparently due to rapid and profound hypothermia, and the dose was reduced to 75 μg/kg. Even this dose still caused occasional fatalities in the −8°C barpress experiments. The same dose proved 100% fatal when used for barpress ex-

TABLE 3

EFFECT OF MAXIMAL THERMOGENIC DOSES OF RO40-2148 AND ICI D7115 METABOLITE ON OPERANT RESPONDING FOR HEAT IN OBESE RATS

	Tin (°C)	Tont (°C)	dT (°C)	BP/min (presses/min)	R/min (s/min)	R/BP (s/press)	dS (kJ)	HI (kJ)	NTB (kJ)	NTE (%)
CON	36.66 ±0.19	38.11 ±0.11	1.45 ±0.19	2.47 ±0.19	17.02 ±0.64	7.10 ±0.41	3.66 ±0.66	50.74 ±2.01	47.08 ±1.94	7.20 ±1.24
RO40	36.41 ±0.08	38.26 ±0.08	1.85 ±0.11	1.97 ±0.15	15.66 ±0.59	8.23 ±0.65	4.76 ±0.47	48.43 ±2.73	43.68 ±2.52	9.84 ±0.81
ICI	36.23 ±0.10	38.20 ±0.09	1.98* ±0.02	1.93 ±0.24	14.22† ±0.67	7.27 ±0.84	5.31* ±0.37	44.78* ±1.81	39.47* ±1.70	11.89† ±0.77

Mean values ± SEM; n = 8; 4 male plus 4 female, obese Zucker rats. *$p<0.05$, †$p<0.01$ vs. CON.
See Table 1 for explanation of column heads.

periments in obese Zucker rats, even when the dose per kg body weight was reduced by 70% to allow for their lower body water content compared to lean rats. This extreme sensitivity precluded the use of ISO in the comparison with RO40, and an additional β_3-agonist (ICI) was therefore substituted in the barpress experiments with obese rats.

The higher dose of RO40 used for Experiments 2–4 (5 mg/kg) was a supramaximal dose, and this was chosen to see if there was any loss of β_3-selectivity, i.e., would the β_1/β_2 effects seen with ISO become apparent at high doses of RO40. The ICI compound (D7114) is maximally thermogenic at a dose of 1 mg/kg, but the compound used for Experiment 4 was the acid metabolite of D7114. The acid metabolite is produced in vivo from the parent compound and is mainly responsible for its adrenergic agonist activity (B. Holloway, ICI Pharmaceuticals, personal communication). The thermogenic potency of the metabolite is similar to D7114 and, therefore, the dose (1.4 mg/kg) of this β_3-agonist was also supramaximal.

The thermoregulatory responses to the novel β_3-agonists were much as expected for thermogenic compounds, i.e., increased colonic temperature in a warm (22°C) environment (Experiment 2), and greater rises in colonic temperature and heat storage in conjunction with reduced requirements for radiant heat, resulting in improved net thermal efficiency in the −8°C barpress environment. RO40 was less effective in obese rats than ICI, and this may have been due to retention of the highly lipophilic RO40 within the subcutaneous fat depots at and around the injection site, whereas ICI was distributed more rapidly. When dosed orally, both drugs are equally effective at raising heat production in normal and genetically obese rats, and both show marked β_3-selectivity and antiobesity effects (15,20).

In spite of the potent thermogenic effects of the β_3-agonists, the thermoregulatory responses were not as dramatic as expected. This, however, ignores the potential contribution of the various physiological and behavioral mechanisms available for the homeostatic control of body temperature, and the possible "trade-off" between defending core temperature and preserving an acceptable skin temperature, as discussed above. Thus it is possible that RO40 rats in their home-cage at 22°C could increase heat loss to compensate for their increased heat production, and explain why colonic temperature was significantly greater than in control rats only during the first hour postinjection, when heat production is rising rapidly to peak values. Casual, and, therefore, not necessarily reliable observations of increased grooming activity in rats receiving RO40 suggest there could have been compensatory increases in heat loss via salivary spreading.

In contrast to the effects of the β_3-agonists, both submaximal and maximal doses of ISO produced effects on thermoregulatory behavior that would suggest it was inhibiting, rather than stimulating heat production. However, it is known from the preliminary dose:response trials that ISO is a full thermogenic agonist. Moreover, in subsequent studies (to be reported elsewhere) it was found that the 75 μg/kg dose of ISO produced increases in heat production of 40–50% at normal room temperatures (20–25°C). Thus the hypothermic effects observed in rats at 22°C (Experiment 2) could not be ascribed to inhibition of thermogenesis, and must have been due to heat loss exceeding heat production. In addition, it is possible that the drop on colonic temperature was exacerbated by changes in tissue blood flow resulting in an expansion of the thermal "core" into the cooler "shell."

The cause of the increased operant responses for heat in ISO-treated rats at −8°C is likely to have been due to the same effects as those discussed above, coupled with the additional thermal drain due to the rat's ventral surface being exposed to the cold environment and shaded from the infrared lamps. In addition, it is possible that ISO does not have a thermogenic response in the extreme cold, and may even inhibit heat production. Such an apparently unlikely suggestion should not be disregarded, since Zylan and Carlisle (31) have found that doses of norepinephrine that are thermogenic at thermoneutral/warm ambient temperatures reduce oxygen consumption below control levels at 5°C. It is not known if ISO responses are affected similarly at these low ambient temperatures, nor what the effect of cold would be on responses to the β_3-agonists, although barpress activity at −8°C suggests that the β_3-agonists are still thermogenic.

Whatever the mechanisms responsible for these unexpected effects of ISO on body temperature and behavioral thermoregulation, they are presumed to be mediated by peripheral β-adrenoceptors, although Dooley et al. (6) have suggested a central action. These workers observed decreases in colonic temperature in mice receiving (±)-isoproterenol, but found more marked decreases when pro-drugs of ISO (i.e., compounds that are metabolised to isoproterenol in vivo) were injected. It was claimed that these pro-drugs could pass the blood-brain barrier and thence exert their central effects following conversion to ISO. The temperature drop following peripheral ISO was assumed to be due to small amounts penetrating the blood-brain barrier, and considering the doses used (30 mg/kg), this would not be too surprising. However, it seems unlikely that penetration of the blood-brain barrier by ISO could occur in the present study, since the doses were three orders of magnitude lower (30–75

μg/kg) than those used by Dooley et al., but were just as effective at decreasing colonic temperature. A central site of action seems even less plausible given reports of hyperthermic responses to central injections of ISO (3, 16, 19). Moreover, Dooley et al. (6) assumed a central action of the ISO pro-drugs, but did not test this experimentally.

On balance, it would seem that these hypothermic effects of ISO are due to effects on peripheral β-adrenoceptors. Furthermore, it is clear that these are almost certainly β_1- and/or β_2-adrenoceptors, since the β_3-adrenoceptor agonists had effects that were predictable for selective thermogenic agonists, and quite opposite to those of ISO. If nothing else, this study has provided behavioral thermoregulatory evidence for the existence of an atypical, or β_3-adrenoceptor to support that derived from in vitro tissue and molecular pharmacological studies. It also provides encouraging support for continuing the development of selective thermogenic agonists for the treatment of obesity and related disorders. The problem of determining the relative importance of β_1- and β_2-adrenoceptor subtypes in mediating the hypothermic effects of ISO is the subject of further investigations, and these will be reported in due course. At this stage, however, it would seem that the hypothermic effects of ISO are likely to be due to its effects on peripheral blood flow, thereby increasing radiant heat loss, although the fact that norepinephrine (a vasoconstrictor) can cause hypothermia (31) suggests that this interpretation might be somewhat facile and simplistic.

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