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The thermogenic actions of α_2-adrenoceptor agonists in reserpinized mice are mediated via a central postsynaptic α_2-adrenoceptor mechanism

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1 The dose-related effects of the selective α_2-adrenoceptor agonists clonidine, UK-14,304 and B-HT 933 on the body temperature of untreated and reserpine-treated mice were investigated.

2 In untreated mice all three agonists induced a dose-related hypothermia. The highest doses of UK-14,304 and B-HT 933, 3 and 100 mg kg^{-1} respectively, elicited a marked (10°C) hypothermia, whereas the maximal hypothermic effect of clonidine (5.5°C) was less pronounced and reached a plateau at a dose of 0.5 mg kg^{-1} i.p.

3 Reserpine (2.5 mg kg^{-1}, s.c.) induced a marked hypothermia in the mouse; 18 h after injection body temperature had decreased to only slightly (0.5–1.5°C) above ambient (19°C).

4 All three α_2-agonists produced a partial dose-related reversal of reserpine-induced hypothermia; maximal thermogenic responses (9–10°C increases in body temperature) were elicited by doses of 0.2, 0.5 and 16 mg kg^{-1} i.p. of clonidine, UK-14,304 and B-HT 933 respectively, and the log dose-response curves for all 3 agonists were bell-shaped.

5 Following intracerebroventricular administration to reserpine-treated mice, the thermogenic response to clonidine was more rapid in onset, and the agonist was 20 fold more potent than when injected i.p.

6 The selective α_2-adrenoceptor antagonists, idazoxan (0.05–0.5 mg kg^{-1}), Wy 26392 (0.3–5.0 mg kg^{-1}) and yohimbine (0.1–1.6 mg kg^{-1}) given orally attenuated the thermogenic responses to all 3 agonists in reserpinized mice in a dose-related manner. Pretreatment with a single dose of idazoxan (0.3 mg kg^{-1}, orally) elicited a 6 fold parallel shift to the right in the dose-response curve to clonidine.

7 The selective α_1-adrenoceptor antagonists, prazosin (10 mg kg^{-1}) and indoramin (3–10 mg kg^{-1}), and the β-adrenoceptor antagonist, propranolol (10 mg kg^{-1}), only partially attenuated the thermogenic responses to the α_2-agonists in reserpinized mice. These effects were variable and not clearly dose-related.

8 Pretreatment of reserpinized mice with the catecholamine synthesis inhibitor, α-methyl-*p*-tyrosine, markedly attenuated (60–95%) the thermogenic response to the noradrenaline uptake inhibitor, desipramine (0.13–12.5 mg kg^{-1}, i.p.), but only slightly reduced (10–35%) that to clonidine (0.032–0.5 mg kg^{-1}, i.p.).

9 These results suggest that α_2-adrenoceptor agonists reverse reserpine-induced hypothermia via a central mechanism involving activation of postsynaptic α_2-adrenoceptors.

Introduction

Recent evidence suggests that α_2-adrenoceptors, similar or identical to the α_2-autoreceptors described by Langer (1974), are not confined to presynaptic sites but are also located on postsynaptic membranes (see review by Timmermans & Van Zwieten, 1981). The majority of the results which led to this conclusion were derived from studies on vascular smooth muscle preparations (Timmermans & Van Zwieten, 1981). It is now apparent, however, that α_2-adrenoceptors are also found at postsynaptic sites

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within the central nervous system. For example, clonidine is thought to produce its hypotensive action and to stimulate growth hormone secretion via activation of central postsynaptic α_2-adrenoceptor mechanisms (Hoefke, 1980; Eriksson *et al.*, 1982). Indeed, radioligand binding studies with [^{3}H]-clonidine after the selective destruction of central noradrenergic neurones by 6-hydroxydopamine imply that the majority of α_2-adrenoceptors within the central nervous system are located postsynaptically rather than presynaptically on noradrenergic nerve terminals (U'Prichard *et al.*, 1979; Dausse *et al.*, 1982).

One of the many pharmacological effects induced by reserpine in rodents is a profound hypothermia (Askew, 1963), which is thought to result from the depletion of central and peripheral neuronal stores of catecholamines (noradrenaline (NA) and dopamine) and 5-hydroxytryptamine (5-HT) (Shore, 1962). In this paper we present evidence suggesting that the thermogenic action of a series of selective α_2-adrenoceptor agonists in reserpine-treated mice is mediated via activation of a central postsynaptic α_2-adrenoceptor mechanism. A preliminary account of this work was presented to the British Pharmacological Society (Bill & Stephens, 1984).

Methods

Animals

Female, albino, T/O strain mice (18–27 g) supplied by Tuck were used. Before experimentation the animals were housed in groups of 15 at an ambient temperature of 21–23°C on a 12 h light/dark cycle (light period 08 h 00 min to 20 h 00 min) with free access to standard laboratory diet (CRM pellets) and tap water.

Reserpine treatment and measurement of body temperature

Experiments were performed on groups of 8 mice per treatment unless otherwise specified. Animals were injected s.c. with 2.5 mg kg^{-1} of reserpine and maintained overnight for 18 h, and for the duration of the subsequent experimental period in plastic cages (30 × 15 × 12 cm) with sawdust in the bottom at an ambient temperature of 19.0 ± 0.5°C.

Body temperatures were measured with a rectal thermistor probe inserted to a standard depth of 2 cm into the colon and a digital thermometer (Comark Electronics Limited, Sussex), while the mice were lightly restrained in the hand by the scruff and the tail.

Intracerebroventricular (i.c.v.) injection techniques

Clonidine or vehicle (sterile isotonic saline) were injected into the lateral cerebral ventricles of reserpine-treated mice (weighing 18–22 g) in a dose volume of 5 μl per mouse according to the method of Brittain (1966). The site of injection was within 1 mm of a point on the midline 2 mm rostral to a line joining the anterior bases of the ears. Injections were made with a sterile 25 μl Hamilton syringe with a 26/10 mm gauge needle inserted perpendicularly through the skull into the brain, and penetrated the brain to a depth of 2.0–2.5 mm below the top surface of the skull. The location of the injection site was verified by injection of 5 μl of a 1 in 10 dilution of Indian ink in isotonic saline. Brains were then removed, sectioned and examined histologically for the presence of particles of ink in the cerebral ventricles. These examinations revealed a success rate for the i.c.v. injections of approximately 90%.

Antagonist studies

Antagonists were administered orally to reserpine-treated mice 17 h after reserpine over the dose ranges shown in Table 1. Thirty minutes later clonidine (0.2 mg kg^{-1}), UK-14,304 (0.4 mg kg^{-1}), B-HT 933 (16 mg kg^{-1}) or vehicle were injected i.p. Body temperatures were recorded immediately before each drug injection and at 1 or 2 h intervals for 6 h, post-injection of the agonists. In all experiments vehicle/vehicle, vehicle/agonist and antagonist/vehicle control groups were included. For each treatment group the mean maximum increase in body temperature over the 6 h period post injection of the agonist was calculated. From these data the percentage inhibition by the antagonists of the response to each agonist was determined and ED_{50} values for the antagonists (doses to inhibit the thermogenic response to each agonist by 50%) together with 95% confidence limits obtained by linear regression analysis.

Catecholamine synthesis inhibition

The effects of pretreatment with the catecholamine synthesis inhibitor, α-methyl-*p*-tyrosine (α-MPT), on the responses of reserpine-treated mice to clonidine and the noradrenaline uptake inhibitor, desipramine, were examined. Groups of 8 mice were dosed i.p. with either vehicle or 300 mg kg^{-1} of α-MPT one hour before the s.c. injection of reserpine (2.5 mg kg^{-1}). A further dose of vehicle or α-MPT (100 mg kg^{-1}) was administered i.p. 16 h after reserpine. Clonidine (0.013–1.25 mg kg^{-1}) and desipramine (0.13–12.5 mg kg^{-1}) were then injected i.p. 2 h after the last dose of α-MPT. Body temperatures

were measured before clonidine and desipramine administration and at 1 h intervals for the following 6 h.

Measurement of brain monoamines and metabolites

The same dosing schedule as that described above was used to determine the effects of reserpine alone and combined treatment with reserpine and α-MPT on brain levels of noradrenaline, dopamine, 5-HT, 3,4-dihydroxyphenylacetic (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA). Whole mouse brain levels of these materials were assayed by high performance liquid chromatography (h.p.l.c.) with electrochemical detection 18 h after injection of reserpine, by the method of Wagner *et al.* (1982).

Experimental design and statistics

In all experiments animals were dosed according to a predetermined sequence such that they were assessed in a balanced order with respect to time and treatment received. Also, with the exception of reserpine, the experimenter was unaware of the treatment(s) that each animal had received.

Body temperatures and temperature changes are shown as mean ± s.e.mean and were analysed by Student's unpaired *t* test. Temperature changes are calculated as the mean maximum increases occurring over the 6 or 8 h period post injection of the agonists or desipramine.

Drugs

The following drugs were used in this study: α-methyl-*p*-tyrosine methylester hydrochloride (Sigma), B-HT 933 ((azepexole), 2-amino-6-ethyl-4,5,7,8-tetrahydro-6H-oxazolo {5,4-d} azepine dihydrochloride, (Boehringer-Ingelheim)), clonidine hydrochloride (Boehringer-Ingelheim), desipramine hydrochloride (Geigy Pharmaceuticals), idazoxan hydrochloride (Reckitt and Colman), indoramin hydrochloride (Wyeth), prazosin hydrochloride (Pfizer), (±)-propranolol hydrochloride (Sigma), reserpine (Sigma), UK-14,304 (5-bromo-6-[2-imidazolin-2-yl-amino]-quinoxaline tartrate, (Pfizer)), Wy 26392 (N-methyl-N-(1,3,4,6,7,11bα-hexahydro-2H-benzo-{a}-quinolizin-2β-yl)-propan-1-sulphonamide hydrochloride, (Wyeth)), yohimbine hydrochloride (Sigma).

For oral administration the antagonists were dissolved or suspended in 0.4% hydroxypropylmethylcellulose (HPMC) in distilled water. Other drugs (except reserpine) were dissolved in isotonic saline for i.p. injection. Reserpine was prepared in a stock solution containing 4% benzyl alcohol, 0.5% citric acid and 20% Tween 80 in distilled water to give a final concentration of reserpine of 5 mg ml^{-1}. This stock solution was diluted 20 fold to give the required dose of reserpine for s.c. injection. All drugs were administered in a dose volume of 10 ml kg^{-1}, apart from i.c.v. injections when the dose volume was 5 μl per mouse. Drug doses in the text refer to the salt.

Results

Effects of α_2-adrenoceptor agonists on the body temperature of non-reserpinized mice

Groups of previously untreated mice were dosed i.p. with either vehicle, clonidine, UK-14,304 or B-HT 933 and body temperatures were recorded before drug injection and at 30 min intervals for the next 2 h.

The mean body temperature of non-reserpinized mice before drug treatment was 37.5 ± 0.2°C (n = 108). Clonidine (0.045–0.5 mg kg^{-1}), UK-14,304 (0.3–3.0 mg kg^{-1}) and B-HT 933 (10–100 mg kg^{-1}) all induced a dose-dependent hypothermia (Figure 1). The hypothermic effects of all three α_2-agonists were rapid in onset, with maximal decreases in body temperature observed at 30–60 min post-injection. After 60 min the body temperature of the drug-treated animals returned gradually towards that of the vehicle-controls. The highest doses of UK-14,304

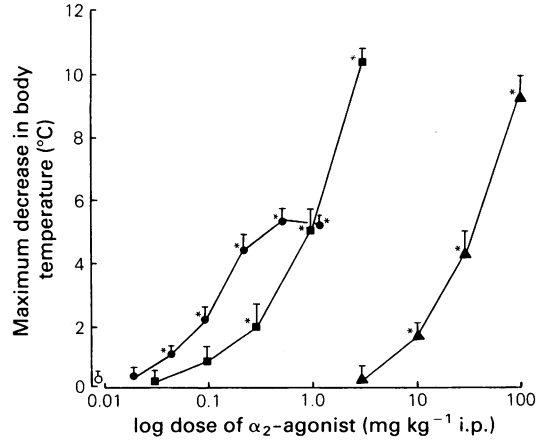


Figure 1 Dose-response relationships for the hypothermia induced by clonidine, UK-14,304 and B-HT 933 in non-reserpinized mice. Groups of 8 mice were dosed i.p. with clonidine (●), UK-14,304 (■), B-HT 933 (▲) or saline (○). Body temperatures were recorded at 30 min intervals for the next 2 h, and the mean maximum decrease in body temperature occurring over this time period calculated for each treatment group. * $P < 0.05$ (Student's unpaired *t* test cf. vehicle (saline) control group). Vertical bars denote ± s.e.mean.

and B-HT 933, 3 and 100 mg kg^{-1} respectively, elicited marked (10–10.5°C) decreases in temperature (Figure 1). In contrast, the maximal hypothermic effect of clonidine (a 5.5°C decrease in body temperature) was less pronounced and reached a plateau at a dose of 0.5 mg kg^{-1} i.p. Clonidine and UK-14,304 were 30–100 fold more potent than B-HT 933 as hypothermic agents.

Effects of reserpine treatment

Reserpine (2.5 mg kg^{-1}, s.c.) evoked a marked and long lasting hypothermia in the mouse. By 18 h after injection the animals were completely immobile, full blepharoptosis was present and body temperatures had decreased to only slightly (0.5–1.5°C) above ambient (19°C), a level of hypothermia that persisted for at least the following 12 h. The s.c. administration of the vehicle for reserpine (10 ml kg^{-1}) had no significant effect on mouse body temperature. At 18 h post-injection the mean body temperature of a group of 8 mice treated with the reserpine-vehicle was 35.5 ± 0.2°C compared to a mean body temperature of 35.8 ± 0.1°C for a group of 8 untreated mice maintained at 19°C for 18 h.

Effects of α_2-adrenoceptor agonists on the body temperature of reserpinized mice

Groups of 8 reserpinized mice were injected i.p. with either vehicle or various doses of clonidine, UK-14,304 or B-HT 933 18 h after reserpine. Body temperatures were recorded prior to drug injection and at 1 or 2 h intervals for the next 8 h.

The i.p. injection of saline alone had no significant effect on body temperature (Figure 2). Clonidine, UK-14,304 and B-HT 933 all evoked a moderate (maximum 10°C) and dose-related reversal of reserpine-induced hypothermia (Figure 3), with lowest doses inducing a significant increase in body temperature of 0.012, 0.02 and 2.5 mg kg^{-1} i.p. respectively. The time-dependent effects of various doses of clonidine (0.032, 0.2 and 1.15 mg kg^{-1}) on the body temperature of reserpinized mice are shown in Figure 2. Similar time-response curves were obtained with appropriate doses of UK-14,304 and B-HT 933. The thermogenic actions of the lower doses of all three agonists were slow in onset, such that the most rapid increases in body temperature were recorded 3–5 h after treatment with peak effects observed at 5–7 h. Maximal thermogenic responses (9–10°C increases in body temperature) were elicited by doses of 0.2, 0.5 and 16 mg kg^{-1} i.p. of clonidine, UK-14,304 and B-HT 933 respectively, and did not differ appreciably in magnitude. Higher doses of the agonists elicited a more even change in temperature with time, but the peak effects were progressively

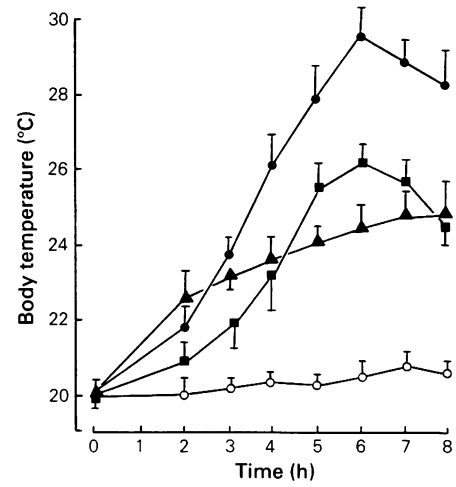


Figure 2 Time-dependent effects of various doses of clonidine on the body temperature of reserpinized mice. Groups of 8 mice were injected i.p. with vehicle (○), or 0.032 (■), 0.2 (●) and 1.15 mg kg^{-1} (▲) of clonidine 18 h after administration of reserpine (2.5 mg kg^{-1}, s.c.). Body temperatures were recorded prior to clonidine and at 1 or 2 h intervals for the next 8 h. The mean body temperature of each treatment group was calculated at each time point. Peak thermogenic responses to 0.032 and 0.2 mg kg^{-1} of clonidine were clearly defined and occurred at 6 h post-injection. Vertical bars denote ± s.e.mean.

smaller, i.e. the dose-response curves for the thermogenic actions of all three agonists were bell-shaped (Figure 3). In molar terms, clonidine and UK-14,304 were approximately equipotent as thermogenic agents, and both drugs were considerably (30–100 fold) more potent than B-HT 933.

Effects of intra-cerebroventricular injection of clonidine on the body temperature of reserpinized mice

Groups of reserpinized mice were injected i.c.v. with sterile isotonic saline or clonidine (0.8–200 μg kg^{-1}). Body temperatures were recorded prior to clonidine and at 1 h intervals for the next 6 h.

The i.c.v. injection of saline alone produced a slight (2–3°C), but statistically significant ($P < 0.05$) increase in the body temperature of reserpinized mice. The dose-related effects of clonidine on body temperature following i.c.v. and i.p. (0.012–1.25 mg kg^{-1}) administration are compared in Figure 4. Central injection of clonidine (0.8–12 μg kg^{-1}) evoked a dose-dependent and marked reversal (maximum effect, 11°C increase) of reserpine-induced hypothermia. This thermogenic effect was more rapid in onset than when the agonist

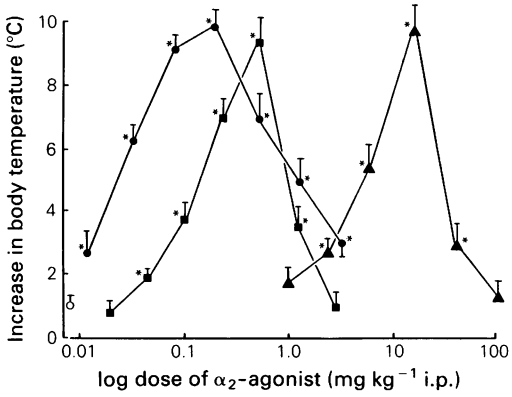


Figure 3 Dose-response relationships for the reversal of reserpine-induced hypothermia by clonidine, UK-14,304 and B-HT 933. Groups of 8 reserpinized mice were injected i.p. with clonidine (●), UK-14,304 (■), B-HT 933 (▲) or saline vehicle (○) 18 h after administration of reserpine (2.5 mg kg^{-1}, s.c.). Body temperatures were recorded prior to the injection of agonist and at 1 or 2 h intervals for the next 8 h. The mean maximum increase in body temperature occurring over this 8 h period was calculated for each dose of the agonist. The dose-response curves for all three agonists were bell-shaped. * $P < 0.05$ (Student's unpaired t test cf. vehicle (saline) control group). Vertical bars denote ± s.e.mean.

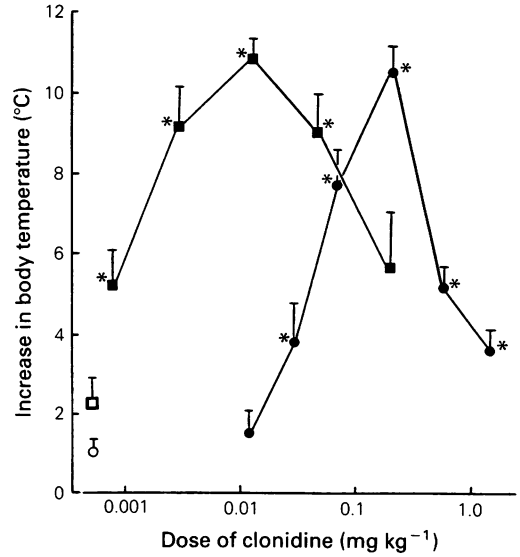


Figure 4 Dose-related effects of clonidine on the body temperature of reserpinized mice following intracerebroventricular (i.c.v.) and i.p. administration. Groups of 8 reserpinized mice were injected i.c.v. with 5 µl per mouse of sterile saline (□) or 0.8–200 µg kg^{-1} of clonidine (■), or i.p. with vehicle (○) or clonidine (0.012–1.25 mg kg^{-1}) (●) 18 h after reserpine (2.5 mg kg^{-1}, s.c.). Body temperatures were recorded at 1 h intervals for the next 6 h. The mean maximum increase in body temperature occurring over this 6 h period was calculated for each treatment group. Clonidine was 20 fold more potent as a thermogenic agent following i.c.v. injection. * $P < 0.05$ (Student's unpaired t test cf. i.c.v. saline or i.p. saline). Vertical bars denote ± s.e.mean.

was given peripherally, with peak increases in body temperature observed at 3–4 h after i.c.v. injection. As when the drug was injected i.p., the dose-response curve for reversal of reserpine-induced hypothermia by i.c.v. clonidine was bell-shaped, with higher doses of clonidine (50–200 µg kg^{-1}) inducing progressively smaller increases in temperature. Comparison of doses to elicit maximal thermogenic responses revealed that clonidine was 20 fold more potent when injected i.c.v. than i.p.

Effects of α- and β-adrenoceptor antagonists on the thermogenic actions of α_2-adrenoceptor agonists in reserpinized mice

The doses of clonidine (0.2 mg kg^{-1}), UK-14,304 (0.4 mg kg^{-1}) and B-HT 933 (16 mg kg^{-1}) used in the antagonist interaction studies were selected on the basis of previous experiments as being doses that would induce near maximal thermogenic responses. The results are summarised in Table 1.

None of the antagonists alone significantly affected the body temperature of reserpinized mice. Pretreatment with the selective α_2-adrenoceptor antagonists, idazoxan (0.05–0.5 mg kg^{-1}), yohimbine (0.1–1.6 mg kg^{-1}) and Wy 26392 (Lattimer *et al.*, 1982) (0.3–5.0 mg kg^{-1}) attenuated the thermogenic responses to all three α_2-agonists in a dose-dependent manner; the ED_{50} values for antagonism of responses to UK-14,304 were 0.15, 0.3 and 1.0 mg kg^{-1} p.o. respectively. Similar doses of these antagonists also inhibited the thermogenic actions of B-HT 933 and clonidine by 50% (Table 1). The rank order of potency of the antagonists, idazoxan > yohimbine > Wy 26392 was the same against all three agonists. In a further experiment, pretreatment of reserpinized mice with idazoxan also attenuated the increase in body temperature elicited by the central injection of clonidine (12 µg kg^{-1}), with an ED_{50} value of 0.1 mg kg^{-1} orally.

The selective α_1-adrenoceptor antagonists, prazosin and indoramin, at doses ⩾3 mg kg^{-1} orally also partially (approx. 50%) inhibited the thermogenic responses to clonidine, UK-14,304 and B-HT 933. These effects, however, were variable, were not clearly dose-related and only reached statistical significance ($P < 0.05$) against UK-14,304 and B-HT 933 at the highest dose of antagonist tested

Table 1 Summary of the effects of selective adrenoceptor antagonists on the thermogenic responses evoked by clonidine, UK-14,304 and B-HT 933 in reserpinized mice

Antagonist	Dose range ($mg\,kg^{-1}$)	*Clonidine* ($0.2\,mg\,kg^{-1}$) Effect	ED_{50}	*UK-14,304* ($0.4\,mg\,kg^{-1}$) Effect	ED_{50}	*B-HT 933* ($16\,mg\,kg^{-1}$) Effect	ED_{50}
Yohimbine (α_2)	0.1–1.6	+++	0.6 (0.4–0.8)	+++	0.3 (0.2–0.4)	+++	0.3 (0.2–0.4)
Idazoxan (α_2)	0.05–0.5	+++	0.25 (0.2–0.3)	+++	0.15 (0.05–0.2)	+++	0.15 (0.1–0.2)
Wy 26392 (α_2)	0.3–5.0	+++	1.2 (0.9–1.6)	+++	1.0 (0.5–2.0)	+++	0.75 (0.5–1.0)
Indoramin (α_1)	0.3–10	++	≃5†	+	>10	+	>10
Prazosin (α_1)	0.3–10	++	≃5†	+	>10	+	>10
Propranolol ($\beta_{1\&2}$)	0.3–10	+	>10	+	>10	+	>10

The antagonists were administered to groups of 8 reserpinized mice orally 30 min prior to the i.p. injection of the agonists. The mean maximum increase in body temperature over the 6 h period after injection of the agonist was calculated for each treatment group and % inhibition by the antagonists determined. (+++) = complete dose-related inhibition, (++) = partial inhibition (approx. 50% inhibition at 2 dose levels), (+) = slight inhibition (significant effect at top dose tested only). For each treatment group the mean maximum increase in body temperature over the 6 h period post injection of the agonist was calculated. From these data the percentage inhibition by the antagonists of the response to each agonist was determined and ED_{50} values (doses to inhibit the thermogenic response to each agonist by 50%) and 95% confidence limits obtained by linear regression analysis. † denotes the ED_{50} value was estimated from data where the effect of the antagonist was not clearly dose-related.

($10\,mg\,kg^{-1}$). Similarly, the β-adrenoceptor antagonist, propranolol, significantly ($P < 0.05$) attenuated (30–50%) the thermogenic responses to the α_2-agonists only at the relatively high dose of $10\,mg\,kg^{-1}$ (Table 1).

Effects of single doses of idazoxan, indoramin and propranolol on the dose-related effects of clonidine in reserpinized mice

In a further series of experiments, groups of reserpinized mice received single doses of either idazoxan ($0.3\,mg\,kg^{-1}$), indoramin ($10\,mg\,kg^{-1}$), propranolol ($7.5\,mg\,kg^{-1}$) or vehicle, orally, 17 h after reserpine. Thirty minutes later saline or a series of doses of clonidine ($0.032–3.1\,mg\,kg^{-1}$) were injected i.p. Body temperatures were recorded before each drug treatment and at 2 h intervals for 6 h post-clonidine. The results of these experiments are summarized in Figures 5a, b and c.

Pretreatment with idazoxan completely abolished the thermogenic responses to low doses ($\leqslant 0.2\,mg\,kg^{-1}$) of clonidine, and produced a 6 fold, parallel shift to the right in the bell-shaped dose-response curve to the drug (Figure 5a). Indoramin pretreatment significantly ($P < 0.05$) reduced the thermogenic responses to all but the lowest dose of clonidine ($0.032\,mg\,kg^{-1}$) by approximately 45–55% (Figure 5b). Pretreatment with propranolol produced a slight attenuation of the responses to all but the highest dose of clonidine ($3.1\,mg\,kg^{-1}$), an effect which reached a significant ($P < 0.05$) level only against a dose of $0.2\,mg\,kg^{-1}$ of the agonist (Figure 5c).

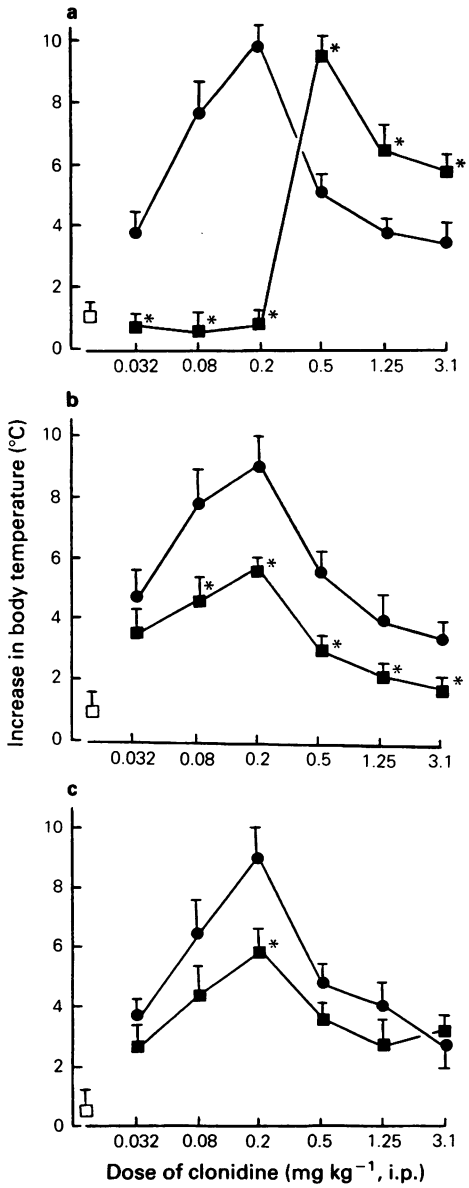
Effects of catecholamine synthesis inhibition on the responses to clonidine and desipramine in reserpinized mice

Desipramine ($0.13–12.5\,mg\,kg^{-1}$ i.p.) evoked a marked (3–13°C) and dose-related increase in the body temperature of mice that had received reserpine alone. Pretreatment with the catecholamine synthesis inhibitor, α-MPT ($300 + 100\,mg\,kg^{-1}$, i.p.), markedly attenuated (60–95%) the thermogenic responses to all doses of desipramine (Figure 6a). In contrast, the thermogenic action of clonidine ($0.032–1.25\,mg\,kg^{-1}$, i.p.) was only slightly decreased (10–35%) by treatment with α-MPT (Figure 6b), an effect which reached statistical significance ($P < 0.05$) only against responses to 0.2 and $0.5\,mg\,kg^{-1}$ of the agonist.

Effects of reserpine and α-methyl-p-tyrosine on brain monoamine and metabolite levels

By 18 h after the injection of reserpine ($2.5\,mg\,kg^{-1}$, s.c.) brain levels of NA and dopamine had decreased to only 5% and 5-HT levels to only 17% of those measured in the brains of vehicle-treated control

mice (Table 2). In contrast, a 130% increase in DOPAC and a 62% increase in 5-HIAA levels were observed 18 h after reserpine. The combined treatment with α-MPT and reserpine further depleted brain levels of NA and dopamine to only 3% of control values, but had no further effect on brain 5-HT levels. Pretreatment with α-MPT did, however, completely attenuate the production of DOPAC (Table 2), indicating that catecholamine synthesis had been almost completely inhibited.



Discussion

The present study demonstrates that clonidine and two highly selective α_2-adrenoceptor agonists, B-HT 933 (Kobinger & Pichler, 1977) and UK-14,304 (Cambridge, 1981), evoke a marked and dose-related reversal of reserpine-induced hypothermia in the mouse. The data presented also suggest that this thermogenic action is mediated through activation of α_2-adrenoceptor mechanisms. Thus, clonidine was a slightly more potent thermogenic agent than UK-14,304, which is in agreement with the relative potencies of the two agonists for inducing mydriasis in the anaesthetized rat (Berridge *et al.*, 1983), an effect thought to be mediated via central α_2-adrenoceptors. The finding that clonidine was approximately 100 fold more potent than B-HT 933 also compares well with estimates of their relative potencies as agonists at α_2-adrenoceptors in cardiovascular preparations obtained by Kobinger & Pichler (1977), who found B-HT 933 to be between 80–150 fold less potent than clonidine. Furthermore, the observation that low doses of the α_2-adrenoceptor antagonists, idazoxan, Wy 26392 (Lattimer *et al.*, 1982) and yohimbine attenuated the thermogenic responses to all three α_2-agonists in a dose-dependent manner also suggests the involvement of α_2-adrenoceptor mechanisms. The rank order of potency of the antagonists, idazoxan > yohimbine > Wy 26392 for antagonism of the responses to the α_2-agonists is in agreement with their relative potencies as antagonists at α_2-adrenoceptors in the pithed rat preparation (Doxey *et al.*, 1983; Paciorek *et al.*, 1984). That the α_2-adrenoceptor antagonists did not attenuate the thermogenic action of the agonists by limiting their passage into the central nervous system, is suggested by the fact that low doses of idazoxan abolished the increase in body temperature produced by central

Figure 5 Effects of single doses of (a) idazoxan (0.3 mg kg^{-1}), (b) indoramin (10 mg kg^{-1}) and (c) propranolol (7.5 mg kg^{-1}) on the thermogenic dose-response relationship for clonidine in reserpinized mice. The antagonists or vehicle were administered orally to groups of 8 mice 17 h after reserpine (2.5 mg kg^{-1}, s.c.); 30 min later saline or clonidine (0.032–3.1 mg kg^{-1}) were injected i.p. Body temperatures were recorded prior to each drug treatment at 2 h intervals and for 6 h post clonidine. Data points represent the mean maximum increase in body temperature over the 6 h period post-clonidine. (●) Represent the dose-related effects of clonidine in reserpine/vehicle pretreated animals and (■) the effect of the agonist in reserpine/antagonist pretreated animals; (□) indicate the effect of the antagonist alone. Idazoxan evoked a parallel shift to the right in the dose-response curve to clonidine. * $P < 0.05$ (Student's unpaired t test cf. vehicle/clonidine treated group). Vertical bars denote ±s.e.mean.

injection of clonidine. In addition, pretreatment of reserpinized mice with a single dose of idazoxan shifted the dose-response curve for reversal of reserpine-induced hypothermia by clonidine to the right in a parallel manner; an indication of competitive pharmacological antagonism at the α_2-adrenoceptor level.

The finding that the selective α_1-adrenoceptor antagonists, prazosin and indoramin, at doses $\geqslant 3\,mg\,kg^{-1}$ orally partially antagonized the thermogenic responses to the α_2-agonists might, at first sight, suggest the involvement of α_1-adrenoceptor mechanisms. This seems unlikely for a number of reasons. First, although neurochemical studies show that clonidine is selective for α_2-adrenoceptors only at doses $<0.1\,mg\,kg^{-1}$ and that higher doses stimulate α_1-adrenoceptors (Anden *et al.*, 1976), the other two α_2-agonists are highly selective agents. Both UK-14,304 and B-HT 933 exhibit a greater than 300 fold selectivity for the α_2- over the α_1-adrenoceptor in isolated tissue preparations (Cambridge, 1981; Rhodes, 1986). Second, the doses at which the α_1-antagonists elicited a partial antagonism of the responses to the α_2-agonists can be considered rather high. Deniard *et al.* (1983) found that prazosin attenuated the reversal of reserpine-induced ptosis by the α_1-agonists, methoxamine and phenylephrine in the mouse over the dose range 0.01–0.1 $mg\,kg^{-1}$, i.p. Prazosin at a dose of $3\,mg\,kg^{-1}$ also completely abolished the hyperactivity induced by the central injection of phenylephrine in the mouse (Heal, 1984).

In the present study, the inhibitory effects of prazosin and indoramin were slight and not clearly dose-related, in contrast to the clearly dose-related and complete antagonism produced by the selective α_2-adrenoceptor antagonists. Also, pretreatment with a single dose of indoramin inhibited the responses to a range of doses of clonidine to a similar degree. Furthermore, at high dose levels indoramin and prazosin have been shown to induce a moderate hypothermia in their own right (unpublished observations). These findings suggest that the partial inhibition by the α_1-antagonists of the thermogenic response to the α_2-agonists in reserpinized mice may

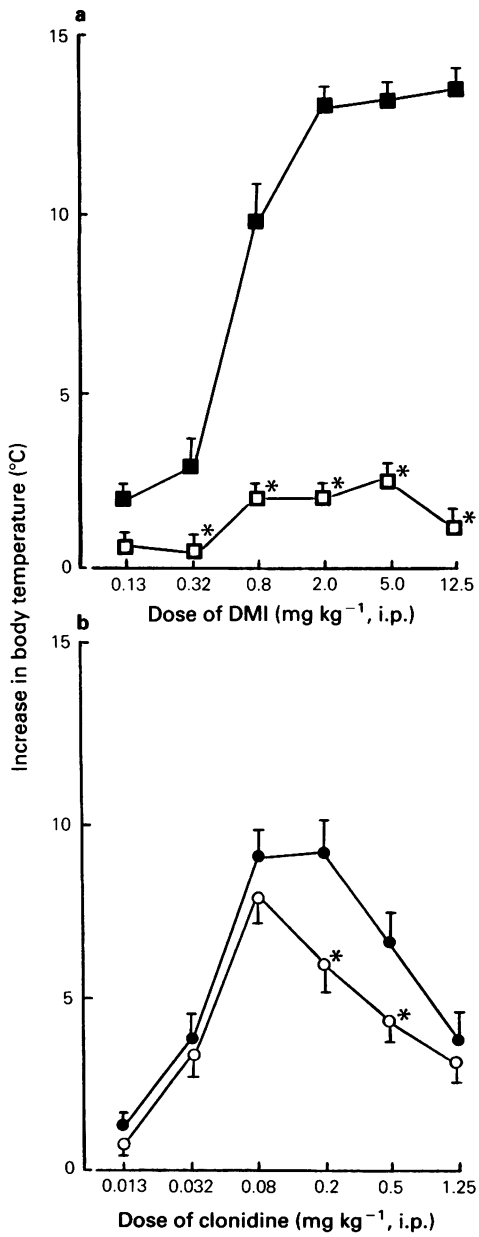


Figure 6 The effects of pretreatment with the catecholamine synthesis inhibitor, α-methyl-*p*-tyrosine (α-MPT) on the thermogenic dose-response relationships to (a) desipramine (DMI) and (b) clonidine in reserpinized mice. Mice ($n = 8$) were dosed i.p. with vehicle or α-MPT ($300\,mg\,kg^{-1}$) 1 h before reserpine ($2.5\,mg\,kg^{-1}$, s.c.). Further doses of vehicle or α-MPT ($100\,mg\,kg^{-1}$) were administered i.p. 16 h after reserpine. DMI (0.13–$12.5\,mg\,kg^{-1}$) and clonidine (0.013–$1.25\,mg\,kg^{-1}$) were injected i.p. 2 h later and body temperatures measured at 1 h intervals for the next 6 h. Solid symbols represent the dose-related effects of DMI and clonidine in animals that received reserpine and vehicle, and open symbols their effects in animals that were treated with reserpine and α-MPT. Pretreatment with α-MPT markedly attenuated the thermogenic responses to all doses of DMI, but only partially attenuated those to 0.2 and $0.5\,mg\,kg^{-1}$ of clonidine. * $P < 0.05$ (Student's unpaired *t* test cf. reserpine alone/DMI or reserpine alone/clonidine treated groups). Vertical bars denote ±s.e.mean.

Table 2 Effects of reserpine and reserpine + α-methyl-*p*-tyrosine treatment on mouse brain monoamine and metabolite levels

Treatments (mg kg^{-1})	*Mouse brain monoamine and metabolite levels* (ng g^{-1} wet weight)				
	NA	*Dopamine*	*DOPAC*	*5-HT*	*5-HIAA*
Vehicle + vehicle	423 ± 16	901 ± 23	107 ± 7	407 ± 20	249 ± 8
Reserpine (2.5 s.c.) + vehicle (i.p.)	22 ± 2*** (−95%)	46 ± 4*** (−95%)	232 ± 11*** (+130%)	69 ± 6*** (−83%)	386 ± 24*** (+62%)
Reserpine (2.5 s.c.) + α-MPT (300 + 100 i.p.)	15 ± 2*** (−97%)	27 ± 2*** (−97%)	ND	92 ± 11*** (−80%)	509 ± 31*** (+114%)

Mice received α-methyl-*p*-tyrosine (α-MPT, 300 mg kg^{-1}, i.p.) or vehicle (saline) 1 h before reserpine (2.5 mg kg^{-1}, s.c.) or vehicle. Further doses of α-MPT (100 mg kg^{-1}, i.p.) or saline were administered 16 h after reserpine. Animals were killed 2 h later (18 h after reserpine) and whole brain levels of monoamines and metabolites assayed as described in the methods section. Reported values are group means ± s.e.mean (n = 5–6). Figures in parentheses represent % change in levels cf. vehicle/vehicle-treated control group.
ND = no DOPAC detected. *** $P < 0.001$ (Student's unpaired t test cf. vehicle-treated control group).

be due to an indirect physiological interaction rather than a direct antagonism at the receptor level. Thus, it is possible that prazosin and indoramin partially opposed the thermogenic effect of the α_2-agonists by blocking α_1-adrenoceptors located on vascular smooth muscle, thereby preventing vasoconstriction and reducing heat conservation.

In the present experiments a dose of 10 mg kg^{-1} of the β-adrenoceptor antagonist, propranolol, also slightly attenuated the thermogenic response to the α_2-agonists. This dose of propranolol may be considered somewhat high for β-adrenoceptor blockade, since Ross (1980) showed that the reversal of reserpine-induced hypothermia in the mouse by clenbuterol, a potent and selective β_2-agonist, was completely abolished by pretreatment with a dose of 2.5 mg kg^{-1}, i.p. of the antagonist. It is therefore possible that propranolol also partially opposed the thermogenic response to the α_2-agonists through a physiological mechanism, i.e. via blockade of β-adrenoceptors located in brown adipose tissue, thereby reducing thermogenesis.

The observation that α_2-adrenoceptor agonists produce a marked increase in the body temperature of reserpinized mice may at first sight be somewhat surprising. In normal laboratory rodents, α_2-agonists induce signs of CNS depression, including overt sedation (Drew *et al.*, 1979), hypoactivity (Delini-Stula *et al.*, 1979) and a moderate to marked hypothermia (Gorka & Zacny, 1981; Colpaert, 1986). These effects are thought to be mediated via stimulation of central and peripheral presynaptic α_2-autoreceptors and a consequent decrease in NA release from noradrenergic nerve terminals (Langer, 1974). Interestingly, clonidine has also previously been reported to inhibit the onset of reserpine-induced hypothermia and ptosis in the mouse when injected prior to reserpine (Gouret *et al.*, 1977). The mechanism responsible for these anti-reserpine effects of clonidine would appear to be stimulation of α_2-autoreceptors. The rate of depletion of intraneuronal stores of catecholamine by reserpine is known to be largely dependent on the degree of neuronal activity (Wakade, 1980). Activation of α_2-autoreceptors by clonidine reduces the rate of firing of noradrenergic neurones (Cedarbaum & Aghajanian, 1977), inhibits NA release and decreases NA turnover, and would therefore be expected to reduce the rate of depletion of NA and inhibit the onset of the behavioural and autonomic signs induced by reserpine. Recent neurochemical studies have confirmed that pretreatment of rats with low doses of clonidine (< 0.2 mg kg^{-1}, i.p.) decreases the rate of depletion of cortical NA by tetrabenazine, a rapidly acting reserpine-like compound (Pettibone *et al.*, 1984).

It seems unlikely, for a number of reasons, that such a presynaptic mechanism is involved in the thermogenic responses to α_2-agonists in animals, in which a marked hypothermia has already been established by the previous administration of reserpine. Firstly, brain NA was markedly depleted (95%) by reserpine prior to the injection of the α_2-agonists. Secondly, stimulation of presynaptic α_2-adrenoceptors might be expected to enhance rather than reverse the hypothermia. Recently, however, the existence of postsynaptic as well as presynaptic α_2-adrenoceptors has been demonstrated in both the periphery and central nervous system (see Timmermans & Van Zwieten, 1981). In fact, radioligand binding studies using [^{3}H]-clonidine suggest that the majority of central α_2-adrenoceptors are located postsynaptically (Dausse *et al.*, 1982).

The observation that pretreatment with the cate-

cholamine synthesis inhibitor, α-MPT, prevented the reversal of reserpine-induced hypothermia by the NA uptake inhibitor, desipramine, is in agreement with the previous work of Sulser & Bickel (1962). This result supports the view that the thermogenic action of desipramine in reserpinized mice is dependent upon the presynaptic-release of newly synthesized NA and the consequent prolongation of the action of the neurotransmitter in the synaptic cleft. In contrast, the thermogenic response to clonidine in reserpinized mice does not appear to require this phenomenon, since treatment with α-MPT had very little effect on the increases in body temperature evoked by the α_2-agonist. This finding suggests that the α_2-adrenoceptor mechanisms involved in mediating the thermogenic action of α_2-agonists are located postsynaptically. Furthermore, centrally injected clonidine was considerably more potent than peripherally administered clonidine at increasing the body temperature of reserpinized mice. This strongly implies that the α_2-adrenoceptors in question are situated in the central nervous system. A recent report has also suggested that the hyperthermia elicited by clonidine in rats maintained at a high ambient temperature is mediated via central postsynaptic α_2-adrenoceptors (Mogilnicka *et al.*, 1985).

The results of the present study provide no clue to the mechanisms beyond the level of the central α_2-adrenoceptor ultimately responsible for producing an increase in the body temperature of reserpinized mice after the administration of α_2-agonists. It may be envisaged that activation of heat production and conservation pathways together with a 'switching off' of heat loss effectors is required. However, the precise nature of the mechanisms involved remains to be determined.

The observation that the dose-response relationships for reversal of reserpine-induced hypothermia by clonidine, UK-14,304 and B-HT 933 were bell-shaped is worthy of comment. Although the mechanism responsible for the down slopes of the dose-response curves is not obvious, the finding that pretreatment with a low dose of the α_2-antagonist, idazoxan, shifted the entirety of the bell-shaped dose-response curve to clonidine to the right, suggests that α_2-adrenoceptor mechanisms may also be involved in this aspect of the response to α_2-agonists. Furthermore, it should be noted that the dose-ranges over which clonidine, UK-14,304 and B-HT 933 induced hypothermia in non-reserpinized mice were almost coincident with the dose-ranges of the down slopes of their dose-response curves in reserpinized mice. Since, the hypothermic response to α_2-agonists in normal laboratory rodents is thought to be mediated via α_2-adrenoceptor stimulation (Gorka & Zacny, 1981), the above findings further imply that the mechanism of the down slopes of the thermogenic dose-response curves to the α_2-agonists in reserpinized mice may also involve α_2-adrenoceptors. Precisely how activation of two different α_2-adrenoceptor mechanisms might have opposing effects on the body temperature of the reserpinized mice appears obscure, and remains the subject of further study.

In summary, we have shown that three selective α_2-adrenoceptor agonists produce a marked reversal of reserpine-induced hypothermia in the mouse. The results of this study strongly suggest that the thermogenic action of these agonists is mediated via activation of a central mechanism involving postsynaptic α_2-adrenoceptors.

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