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Desipramine and nortriptyline antagonize apomorphine and reserpine hypothermia by a different mechanism

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Abstract—The reversal of hypothermia, induced by reserpine or by a high (16 mg) dose of apomorphine, in male Swiss mice, does not seem to utilize a common mechanism. Desipramine (20 mg kg^{-1} i.p., 60 min) or nortriptyline (8 mg kg^{-1} i.p., 60 min) increased temperature in both reserpine (2·5 mg kg^{-1} s.c., 18–19 h) and apomorphine (16 mg kg^{-1} s.c., 30 min) treated mice. In apomorphine-treated animals the effect of both drugs was reversed by the mixed dopaminergic D_1- D_2-antagonist haloperidol (1 mg kg^{-1} i.p., 90 min), the D_1-receptor blocking drug SCH 23390 (0·05 mg kg^{-1} s.c., 30 min), the α_1-adrenoceptor blocking drugs prazosin (3 mg kg^{-1} s.c., 90 min) and phenoxybenzamine (20 mg kg^{-1} i.p., 65 min), the β-adrenoceptor blocking drug (±)-propranolol (10 mg kg^{-1} i.p., 120 min), and the opioid antagonist naloxone (2 mg kg^{-1} i.p., 15 min). In contrast the selective D_2-antagonist (±)-sulpiride (100 mg kg^{-1} i.p., 90 min), and the α_2-antagonist yohimbine (2 mg kg^{-1} i.p., 75 min), failed to effect the reversal of apomorphine hypothermia brought about by desipramine or nortriptyline. Their temperature effects in reserpinized mice were not modified by any of the antagonists tested.

Although no laboratory test mimics human depression, the antagonism of apomorphine- or reserpine-induced hypothermia in mice has been widely used to detect antidepressants (Askew 1963; Puech et al 1978; Przegalinski et al 1980; Francès & Simon 1985).

Apomorphine and reserpine appear to act through opposite neuronal mechanisms, apomorphine stimulating (Ernst 1967) and reserpine inhibiting (Lee 1983) the catecholaminergic system. Therefore it appeared worthwhile to determine the mechanism(s) by which antidepressants known to affect noradrenaline uptake (Ross & Renyi 1967; Iversen 1974; Quinaux et al 1982), such as desipramine and nortriptyline, antagonize the induced hypothermias. This was done by determining the effects of desipramine or nortriptyline on apomorphine and reserpine hypothermia in the presence of noradrenaline (NA) antagonists. Dopamine (DA) is involved in the reversal of reserpine-induced hypothermia (RIH) (Volterra et al 1988) and apomorphine-induced hypothermia (AIH) (Menon et al 1984) and in the mechanism of action of antidepressants (Fekete et al 1980; Borsini et al 1985; Plaznik & Kostowski 1987). In addition, interactions between opiates and DA on body temperature have been reported (Quock 1977; Weiss et al 1984). Therefore the effect of DA antagonists and naloxone on desipramine or nortriptyline activity on AIH and RIH was also studied.

Although Pawlowski & Mazela (1986), have shown that prazosin, phenoxybenzamine and (−)-propranolol antagonized the effect of desipramine on AIH we repeated those experiments using our conditions.

Materials and methods

Animals. Male Swiss albino mice (Nossan, Italy), 20–30 g, were housed in transparent Makrolon cages (42 × 27 × 15 cm) 15 to a cage under controlled temperature (22 ± 1 C) and humidity (55 ± 5%) and a 12 h light dark cycle (lights on 06:00 h) with free access to food and water. They were adapted to the environment for at least 5 days before use. Eight to 11 mice were assigned to treatments on a randomized schedule (Borsini 1985).

Drugs. Apomorphine HCl (Sigma, St. Louis, MO, USA) was dissolved in 1% w/v L-ascorbic acid in distilled water. Reserpine (Serva, Heidelberg, Germany) was first ground in a mortar and then dissolved in 2% w/v L-ascorbic acid in distilled water (Cox & Tha 1975). Desipramine (Nortimil, Chiesi, Parma, Italy), nortriptyline HCl (Recordati, Milan, Italy), prazosin HCl (Ricerchimica, Milan, Italy), (±)- propranolol HCl (Sigma, St. Louis, MO), (±)-sulpiride (Dobren, Ravizza, Milan, Italy) and naloxone HCl (Sigma, St. Louis, MO) were dissolved in 0·9% NaCl (saline). Yohimbine HCl (Aldrich, Milan, Italy) and SCH 23390 ((*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1-H-3-benzazepine 7-ol maleate) (Schering, Bloomfield, NJ, USA) were dissolved with a minimal quantity of 0·1 M HCl and diluted in saline after neutralizing with $NaHCO_3$. Phenoxybenzamine (SK&F, Milan, Italy) was dissolved with a minimal quantity of glacial acetic acid and diluted in saline after neutralizing with $NaHCO_3$. Haloperidol (Lusofarmaco, Milan, Italy) was suspended in a vehicle of NaCl 0·9%, carboxymethylcellulose 0·5%, Tween 80 0·4% in distilled water. All drugs and vehicles were administered in a volume of 10 mg kg^{-1}.

Experimental protocol. Apomorphine 16 mg kg^{-1} (Puech et al 1981) and reserpine 2·5 mg kg^{-1} (Volterra et al 1988) were administered subcutaneously 30 min and 18–19 h, respectively, before rectal temperature measurement. Temperature measurements were made only at the time of peak activity. Experiments were carried out between 9:00–12:30 h. Drugs were administered at doses, routes and time (before recording rectal temperature) reported to exert their maximum activity on the appropriate catecholaminergic or opioid system: prazosin 3 mg kg^{-1} s.c., 90 min (Andén et al 1978), SCH 23390, 0·05 mg kg^{-1} s.c., 30 min (Christensen et al 1984), yohimbine 2 mg kg^{-1} i.p., 75 min (Desiles & Rips 1980), phenoxybenzamine 20 mg kg^{-1} i.p., 65 min (Andén & Strombom 1974), (±)- propranolol 10 mg kg^{-1} i.p., 120 min (Bill et al 1989), (±)-sulpiride 100 mg kg^{-1} i.p., 90 min (Vasse et al 1985), haloperidol 1 mg kg^{-1} i.p., 90 min (Kulkarni 1980), naloxone 2 mg kg^{-1} i.p., 15 min (Moon et al 1980), desipramine 20 mg kg^{-1} i.p., 60 min (Buckett & Diggory 1983) and nortriptyline 8 mg kg^{-1} i.p., 60 min (Puech et al 1981).

Each experimental trial consisted of 4 groups: vehicle + vehicle, antagonist + vehicle, vehicle + antidepressant and antagonist + antidepressant.

Measurement of rectal temperature. Rectal temperature was measured by an Ellab digital thermometer to the nearest 0·1 C, by inserting a thermistor probe 2·5 cm into the rectum of mice hand held only near the base of the tail and otherwise free to move.

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Table 1. Effects of various antagonists on desipramine (DMI) and nortriptyline (NOR) induced reversal of 16 mg kg^{-1} apomorphine hypothermia in the mouse. Data are means ± s.e. of 8–11 mice. Prazosin and SCH 23390 were administered by the subcutaneous route 90 and 30 min, respectively, before rectal temperature readings. Yohimbine, phenoxybenzamine, (±)-propranolol, (±)-sulpiride, haloperidol and naloxone were administered by the intraperitoneal route 75, 65, 120, 90, 90 and 15 min respectively before temperature readings. DMI and NOR were administered by the intraperitoneal route 60 min before temperature readings. Apomorphine was administered by the subcutaneous route 30 min before temperature readings.

Treatment	Dose mg kg^{-1}	Rectal temp. °C Vehicle	DMI	Vehicle	NOR
Vehicle	—	33·9 ± 0·6	38·5 ± 0·3**	34·0 ± 0·2	37·0 ± 0·4**
Prazosin	3	32·4 ± 0·3**	34·3 ± 0·6[a]	32·2 ± 0·2**	33·3 ± 0·2[b]
Vehicle	—	34·1 ± 0·3	38·3 ± 0·4**	34·1 ± 0·3	37·8 ± 0·3**
Phenoxybenzamine	20	33·4 ± 0·2	36·2 ± 0·4[a]	33·4 ± 0·2	35·6 ± 0·6[a]
Vehicle	—	32·4 ± 0·5	35·9 ± 0·5**	32·4 ± 0·5	36·2 ± 0·5**
Yohimbine	2	32·0 ± 0·3	34·0 ± 0·5 n.s.	32·0 ± 0·3	34·9 ± 0·7 n.s.
Vehicle	—	33·7 ± 0·5	37·7 ± 0·3**	33·7 ± 0·5	37·3 ± 0·5**
(±)-Propranolol	10	32·8 ± 0·4	34·7 ± 0·5[a]	32·8 ± 0·4	33·9 ± 0·6[a]
Vehicle	—	33·4 ± 0·4	36·7 ± 0·6*	32·7 ± 0·4	37·2 ± 0·4**
(±)-Sulpiride	100	33·8 ± 0·7	38·3 ± 0·3 n.s.	34·0 ± 0·3*	38·0 ± 0·3 n.s.
Vehicle	—	32·8 ± 0·3	36·9 ± 0·3**	32·8 ± 0·3	36·3 ± 0·5**
SCH 23390	0·05	34·0 ± 0·3*	36·8 ± 0·3[a]	34·0 ± 0·3*	35·9 ± 0·4[a]
Vehicle	—	34·4 ± 0·4	37·5 ± 0·2**	34·4 ± 0·4	37·9 ± 0·4**
Haloperidol	1	34·4 ± 0·2	35·6 ± 0·3[b]	34·4 ± 0·2	35·2 ± 0·2[b]
Vehicle	—	32·6 ± 0·3	37·6 ± 0·4**	32·6 ± 0·3	37·6 ± 0·2**
Naloxone	2	33·4 ± 0·2	36·9 ± 0·3[a]	33·4 ± 0·2	36·8 ± 0·3[b]

ANOVA (2 × 2): [a] = $P < 0·05$; [b] = $P < 0·01$; n.s. = not significant. Tukey's Test: * $P < 0·05$; ** $P < 0·01$ versus appropriate control.

Statistical analysis. Results were evaluated by factorial analysis of variance followed by Tukey's test after testing for homogeneity of variance. Values represent means ± s.e.m. of the rectal temperature readings.

Results

Neither desipramine nor nortriptyline affected the baseline temperature in control animals (data not shown) but both antagonized AIH and RIH. In the apomorphine-treated animals, the antagonistic effects of both antidepressants on hypothermia was significantly reduced by prazosin, phenoxybenzamine, propranolol, SCH 23390 and naloxone but not by yohimbine or sulpiride (Table 1). Significant reversal of desipramine or nortriptyline effects, respectively, by the antagonists at doses shown in Table 1 were as follows: prazosin $F(1,30) = 6·49$ $P < 0·05$, $F(1,40) = 18·40$ $P < 0·01$; phenoxybenzamine $F(1,35) = 5·76$ $P < 0·05$, $F(1,36) = 5·33$ $P < 0·05$; propranolol $F(1,36) = 5·22$ $P < 0·05$, $F(1,35) = 4·79$ $P < 0·05$; SCH 23390 $F(1,38) = 6·01$ $P < 0·05$, $F(1,37) = 4·93$ $P < 0·05$; haloperidol $F(1,36) = 12·33$ $P < 0·01$, $F(1,36) = 17·53$ $P < 0·01$; naloxone $F(1,36) = 4·12$ $P < 0·05$, $F(1,36) = 7·57$ $P < 0·01$. None of the antagonists reduced the antidepressants' effects in reserpinized mice (Table 2). In mice not receiving the antidepressants, the degree of AIH was further decreased by prazosin, increased by SCH 23390 and inconsistently affected by sulpiride, and the degree of RIH was further decreased by yohimbine (Tables 1, 2).

Discussion

Prazosin, propranolol, SCH 23390, haloperidol and naloxone significantly reduced the rise in temperature induced by desipramine or nortriptyline only in AIH, not in RIH. This suggests that both drugs act through a different mechanism in antagonizing apomorphine or reserpine-induced hypothermia in mice.

Our data with prazosin, phenoxybenzamine and propranolol on AIH confirm the results of Pawlowski & Mazela (1986). We have no explanation for the failure of noradrenergic blockers to antagonize the reversal of RIH by the antidepressants. Body temperature in animals after reserpine is lower than after apomorphine. The reduced antagonistic activity of desipramine or noradrenaline on RIH in comparison with that on AIH is consistent with the fact that the efficacy on NA uptake decreases with reduction in body temperature (Hendley et al 1977). However, the ability of prazosin to bind to its receptor sites after reserpinization remains unchanged (Pilc et al 1988); this indicates that prazosin should still exert its antagonistic activity after reserpinization.

In contrast to our results with reserpinized mice, Jori et al (1966) found phenoxybenzamine and propranolol active in antagonizing both antidepressants in reserpinized rats. Species differences could account for this discrepancy.

As for NA receptor blockers, DA receptor antagonists also affected antidepressant-induced reversal of AIH but not RIH.

Haloperidol (D_1-/D_2-antagonist) and SCH 23390 (D_1-antagonist), unlike sulpiride (D_2-antagonist), reduced desipramine and nortriptyline induced temperature increase after AIH; this leads us to hypothesize that D_1-receptor effects are involved in this reversal.

Data in the literature indicate that SCH 23390 binding is not affected by reserpinization (Nisoli et al 1988), therefore its lack of effect on the antidepressants' action in reserpinized mice would not be due to alteration of receptor binding capacity.

As observed with DA and NA antagonists, the opiate antagonist naloxone also antagonized desipramine and nortriptyline effects in apomorphine but not reserpine treated mice.

The fact that antagonists acting on different receptors lead to reversal of the antidepressant effect on AIH could indicate that a multireceptor mechanism is involved in that effect. The possibility that the antagonists activity could be due to non-specific effects seems less likely, for then a similar behaviour of the same antagonists should be seen for all antidepressants. We have data (results not shown) that this is not the case for imipramine and for a research compound with an antidepressant profile of

Table 2. Effects of various antagonists on desipramine (DMI) and nortryptiline (NOR) induced reversal of 2·5 mg kg^{-1} reserpine hypothermia in the mouse. Data are means ± s.e. of 10–11 mice. Reserpine was administered by the subcutaneous route 17/18 h before rectal temperature readings. For administration of antagonists and DMI and NOR see Table 1.

Treatment	Dose mg kg^{-1}	Rectal temp. (°C) Vehicle	DMI	Vehicle	NOR
Vehicle	—	30·6±0·4	33·5±0·3**	31·7±0·4	35·1±0·3**
Prazosin	3	29·8±0·4	31·3±0·5 n.s.	30·8±0·4	33·2±0·4 n.s.
Vehicle	—	29·1±0·6	33·1±0·4**	32·4±0·6	34·2±0·4**
Yohimbine	2	27·6±0·7*	32·8±0·5 n.s.	30·4±0·5**	32·6±0·4 n.s.
Vehicle	—	32·4±0·6	34·0±0·5*	32·4±0·6	34·4±0·4**
(±)-Propranolol	10	31·6±0·6	33·2±0·3 n.s.	31·6±0·6	33·3±0·6 n.s.
Vehicle	—	32·1±0·5	34·1±0·4**	32·1±0·5	34·2±0·3**
(±)-Sulpiride	100	30·8±0·7	34·6±0·3 n.s.	30·8±0·7	34·7±0·4 n.s.
Vehicle	—	30·6±0·5	33·9±0·4**	30·6±0·5	34·2±0·3**
SCH 23390	0·05	30·1±0·5	33·8±0·2 n.s.	30·1±0·5	33·8±0·3 n.s.
Vehicle	—	32·2±0·6	34·3±0·2**	32·7±0·2	34·3±0·3**
Haloperidol	1	32·0±0·5	32·6±0·3 n.s.	31·4±0·5*	33·9±0·2 n.s.
Vehicle	—	31·9±0·5	33·9±0·3**	31·9±0·5	34·6±0·4**
Naloxone	2	31·9±0·5	33·5±0·5 n.s.	31·9±0·5	33·6±0·8 n.s.

ANOVA (2 × 2): n.s. = not significant. Tukey's Test: * $P<0·05$; ** $P<0·01$ versus appropriate control.

activity, which we are currently studying. What seems important is the fact that no single effect is sufficient to account for the antidepressant compounds activity.

In conclusion, the present study shows that AIH but not RIH, is reversed by desipramine and nortriptyline by mechanisms which seem to involve α_1- and β-noradrenergic, dopaminergic D_1- and opiate receptors.

The involvement of DA mechanisms in antidepressant induced reversal after acute administration in AIH is a novel finding, since both drugs have virtually no activity on dopamine uptake and release and receptor binding (Richelson 1987) both in-vitro and in-vivo.

Hypothermia reduction in the reserpine test seems, instead, to implicate non-catecholaminergic mechanisms.

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In-vitro antibacterial activity of noxythiolin and taurolidine

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Abstract—The minimum inhibitory concentrations (MIC) of noxythiolin and taurolidine were determined for strains of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Tests were performed in broth alone and in broth plus 25% v/v serum or 25% v/v urine. Inoculum density was either 10^3, 10^5 or 10^7 colony forming units per mL^{-1}. Slight inoculum-dependent variation in the activity of both agents was observed for some, but not all, strains of *P. aeruginosa* and *S. aureus*. A more pronounced medium-dependent increase in activity was observed with both drugs, with up to 8-fold reduction of values for MIC when tested in the presence of serum or urine. These observations may help to clarify the disparity between the observed clinical efficacy of these agents and relatively poor in-vitro activity when tested using conventional methods in synthetic media.

Noxythiolin (*N*-methyl-*N'*-hydroxymethylthiourea) (Noxyflex S. Geistlich Sons Ltd, Chester), and the related compound taurolidine (bis [1,1-dioxoperhydro-1,2,4-methylene thiadiazinyl-4]methane) (Taurolin, Geistlich Sons Ltd, Chester, UK), are broad spectrum antimicrobials with activity against Gram-negative and Gram-positive bacteria and fungi (Reeves & Schweitzer 1974; Gorman et al 1985).

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The clinical efficacy of noxythiolin (Browne & Stoller 1970) and of taurolidine (Browne et al 1977) correlates poorly with the high minimum inhibitory concentrations (MIC) observed for commonly occurring bacterial pathogens (Reeves & Schweitzer 1974; Browne et al 1977; Brearley & George 1980). Conventional techniques used for the in-vitro study of antibiotic action may not fully reflect the in-vivo activity of these agents. In this report, the in-vitro activity of noxythiolin and taurolidine, determined in broth alone, is compared with the activity observed in the presence of serum or urine.

Materials and methods

Serial two-fold dilutions of noxythiolin and taurolidine were prepared over the range 16 384 to 2 mg L^{-1} in nutrient broth (CM1, Oxoid Limited, Basingstoke) and dispensed in 100 μL volumes to sterile microdilution trays. Separate broth blanks containing no test drug were also prepared for each test strain.

Inocula were prepared by diluting overnight broth cultures of *Staphylococcus aureus* NCTC 6571, *Escherichia coli* NCTC 10418 and *Pseudomonas aeruginosa* NCTC 10662, together with five clinical isolates of each of these species. For noxythiolin, inocula were adjusted to give final inoculum densities of 10^3, 10^5 and 10^7 colony forming units (cfu) mL^{-1}. For taurolidine the inoculum density was 10^5 cfu mL^{-1}. Samples of each inoculum suspension (100 μL) prepared in nutrient broth, were added to each well of the microdilution trays.