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Comparison of the effects of binodaline and amitriptyline on peripheral autonomic functions in healthy volunteers

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1 Twelve healthy male volunteers participated in five experimental sessions separated by weekly intervals. At the beginning of each session the subjects received one single oral dose of the following drugs, according to a double-blind, balanced cross-over design: binodaline hydrochloride (50 mg or 100 mg); amitriptyline hydrochloride (50 mg or 100 mg); lactose placebo.
2 Salivation and resting pupil diameter were assessed before and 2 h after the ingestion of the drugs; baseline sweating, carbachol- or phenylephrine-evoked sweating were measured 2 h following drug taking.
3 Binodaline, like placebo, had little effect on salivary output, whereas amitriptyline caused a dose-dependent decrease in salivation.
4 None of the drugs caused any significant change in resting pupil diameter or in baseline sweating.
5 Carbachol-evoked sweating did not differ significantly following the ingestion of binodaline or placebo; on the other hand responses to carbachol were significantly reduced following amitriptyline.
6 Phenylephrine-evoked sweating was reduced by both binodaline and amitriptyline.
7 The lack of effect of binodaline on salivation, resting pupil diameter, baseline and carbachol-evoked sweating is in agreement with the results of animal experiments indicating the lack of an interaction of this drug with cholinergic mechanisms. The reduction in phenylephrine-evoked sweating would be indicative of an α-adrenoceptor blocking property of this drug.

Keywords binodaline amitriptyline pupil diameter salivation sweat glands carbachol phenylephrine

Introduction

Binodaline (RU-39780), 1-(ω-dimethylamino-ethylmethyl)-amino-3-phenylindole (Figure 1), is a recently developed phenylindole derivative (Schatz *et al.*, 1980) with activities very similar to those of established tricyclic antidepressants in animal tests used to detect potential antidepressant activity. Like imipramine, binodaline antagonizes reserpine- and tetrabenazine-induced ptosis in rats and hypothermia in mice, antagonizes apomorphine-induced hypothermia, and potentiates yohimbine-induced toxicity (Adrian *et al.*, 1981; Jahn *et al.*, 1983; Maj *et al.*, 1983). Furthermore, like imipramine, binodaline inhibits the uptake of noradrenaline, dopamine and 5-hydroxytryptamine into cerebral synaptosomes (Benfield & Luscombe,

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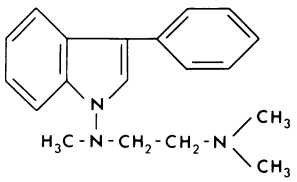

Figure 1 Molecular structure of binodaline.

1983). However, in contrast to established tricyclic antidepressants, binodaline is devoid of antimuscarinic, antihistamine and anti-5-hydroxytryptamine properties (Adrian *et al.*, 1981; Jahn *et al.*, 1983; Maj *et al.*, 1983). Initial clinical trials support the prediction from the preclinical studies that binodaline may be a clinically effective antidepressant (Faltus *et al.*, 1981).

Although observations in the animal laboratory can be used as predictors of the possible autonomic effects of drugs in humans, it is necessary to establish whether the predicted effects occur in humans treated with clinically relevant doses of the drug in question. In the present study, we examined the effects of binodaline on some baseline autonomic functions (salivation, resting pupil diameter, baseline sweat gland activity), and on tissue responses to cholinoceptor and adrenoceptor stimulants (carbachol- and phenylephrine-evoked sweating). The effects of binodaline were compared with those of placebo and of amitriptyline, an antidepressant with well-documented effects on the autonomic nervous system (Ghose *et al.*, 1974, 1976; Peck *et al.*, 1979; Szabadi *et al.*, 1980, 1981).

Methods

Subjects

Twelve healthy male volunteers (aged 19–27 years) participated in the study. All the subjects were informed about the nature and possible risks of the experiment, and all gave written consent. The study was approved by the Ethical Committee of Withington Hospital, Manchester.

Drugs

Two doses (50 mg and 100 mg) of binodaline hydrochloride (Roussel Laboratories), two doses (50 mg and 100 mg) of amitriptyline hydrochloride, and one dose of lactose placebo were taken by each subject. The two single doses of the two antidepressants were comparable to those used in the clinical situation (amitriptyline hydrochloride: see British National Formulary, 1984; binodaline hydrochloride: see Faltus *et al.*, 1981). The drugs were prepared in identical capsules for double-blind administration; each session involved the ingestion of two capsules.

Tests

Salivation This was measured by placing cotton wool rolls in the mouth and assessing the increase in weight during a 1 min period (Peck, 1959). Three dental rolls (size 2) were used in the present experiment (two rolls were placed bucally and one sublingually). The test was repeated three times at 5 min intervals, and the mean of the three assessments was taken as a measure of salivary output (Szabadi *et al.*, 1980).

Pupil diameter This was measured using a photographic technique (Sneddon & Turner, 1969). The pupils were photographed four times at 5 min intervals, and the mean resting pupil diameter was calculated from measurement of four photographs (Szabadi *et al.*, 1980).

Sweating Sweat gland activity on the forearm was assessed using a plastic paint method (Clubley *et al.*, 1978) as described previously (Maple *et al.*, 1982; van den Broek *et al.*, 1984). Spontaneous sweat gland activity was first assessed, and then sweating was evoked by six intradermal injections of either carbachol chloride (six subjects) or phenylephrine hydrochloride (six subjects). The concentrations used were 5, 10, 50, 100, 500 and 1000 μM; the volume injected was 0.05 ml. Drug-evoked sweating was measured 2 min after the injection of carbachol chloride, and 30 s after the injection of phenylephrine hydrochloride (Maple *et al.*, 1982).

Experimental design

Each subject participated in five sessions at weekly intervals. Drugs were allocated to subjects and sessions in a double-blind fashion according to a balanced crossover design. At the beginning of each experimental session, the subject was allowed a 30 min acclimatization period (temperature, lighting conditions, etc.), after which the pre-drug tests (salivation, resting pupil diameter) were carried out. The subjects then ingested the drug capsules, and 2 h later the post-drug tests (salivation, resting pupil diameter, baseline sweating, carbachol- or phenylephrine-evoked sweating) were carried out. The time-course of the session was based on pharmacokinetic data: peak plasma levels of

amitriptyline are attained 2 h after ingestion of a single dose (Jørgensen, 1975), and a similar figure has been quoted for binodaline (Roussel Laboratories: unpublished data).

Analysis of data

For the salivation and resting pupil diameter data, the mean percentage difference between pre-drug and post-drug values was calculated for the whole group of subjects and for each of the five drug conditions. Student's *t*-test was used to compare statistically the pre-drug and post-drug values. The group mean value of baseline sweating obtained after each antidepressant treatment was compared with the group mean value obtained following placebo, using Student's *t*-test. Sweat gland responses to carbachol and phenylephrine were determined by subtraction of the number of active sweat glands detected in the absence of the drug ('baseline sweating') from the total number of active glands detected following the injection of the drug. Carbachol- and phenylephrine- evoked sweating were analyzed separately using two-factor analyses of variance with logarithmically transformed data. The two factors were treatment condition and dose of intradermally injected drug.

Results

The effects of the drugs on salivation and resting pupil diameter are shown in Table 1. Amitriptyline caused a dose-dependent and statistically significant reduction in salivary output, while binodaline and placebo had no significant effect on this measure. Resting pupil diameter was not affected significantly by any of the treatments.

Tables 2 and 3 show the effects of the systemically administered drugs on baseline sweating and on sweat gland activity evoked by increasing doses of carbachol or phenylephrine. Baseline sweating measured following the administration of the antidepressants did not differ from the value obtained following placebo for the whole group of 12 subjects studied (Student's *t*-test: $P > 0.1$ in each case). Analysis of variance revealed that the number of sweat glands activated by either carbachol or phenylephrine was significantly related both to the dose of the intradermally injected drug and systemic treatment condition (Table 4). Individual comparisons between systemic treatments (Student's *t*-test with Dunnett's correction for multiple comparisons) showed that carbachol-evoked sweating was reduced by amitriptyline 50 mg ($P < 0.05$) and 100 mg ($P < 0.005$), but

Table 1 Percentage change from pretreatment level (mean ± s.e. mean)

	Placebo	*Binodaline*		*Amitriptyline*	
		50 mg	*100 mg*	*50 mg*	*100 mg*
Salivation ($n = 12$)	+7.9 ± 7.9	−0.4 ± 10.2	−13.0 ± 6.1	−46.6 ± 9.6*	−66.2 ± 4.8*
Resting pupil diameter ($n = 6$)	+6.2 ± 4.0	+11.9 ± 5.6	+3.8 ± 3.2	−0.9 ± 7.5	−7.8 ± 9.0

*$P < 0.001$ (Student's *t*-test). n = number of subjects studied.

Table 2 Number of sweat glands counted following the intradermal injection of carbachol chloride (0.05 ml)

Concentration of carbachol chloride (μM)	*Placebo*	*Systemically administered drug* *Binodaline*		*Amitriptyline*	
		50 mg	*100 mg*	*50 mg*	*100 mg*
0	10.3 ± 1.8	12.9 ± 3.5	13.3 ± 2.6	13.8 ± 2.3	8.3 ± 1.4
5	78.7 ± 19.0	75.0 ± 17.2	75.3 ± 15.6	63.8 ± 13.4	65.3 ± 12.5
10	112.7 ± 28.0	85.3 ± 13.2	76.3 ± 11.4	83.8 ± 22.0	63.2 ± 18.5
50	171.2 ± 46.4	122.2 ± 23.5	141.7 ± 20.6	101.7 ± 14.2	65.3 ± 13.5
100	149.5 ± 46.7	136.5 ± 43.4	150.2 ± 21.0	138.2 ± 33.7	91.5 ± 24.5
500	281.0 ± 66.2	262.8 ± 48.7	238.2 ± 54.4	148.2 ± 31.4	176.8 ± 34.0
1000	361.3 ± 98.0	329.0 ± 63.9	296.7 ± 53.9	249.8 ± 45.0	250.7 ± 47.4

Values are mean ± s.e. mean obtained in six subjects. Values obtained in the absence of carbachol chloride represent 'baseline sweating'.

Table 3 Number of sweat glands counted following the intradermal injection of phenylephrine hydrochloride (0.05 ml)

Concentration of phenylephrine hydrochloride (μM)	*Placebo*	*Systemically administered drug*			
		Binodaline		*Amitriptyline*	
		50 mg	*100 mg*	*50 mg*	*100 mg*
0	12.5 ± 2.2	16.0 ± 1.9	9.9 ± 1.3	10.5 ± 2.1	16.1 ± 2.5
5	20.2 ± 2.6	23.5 ± 2.3	15.8 ± 2.9	19.3 ± 4.5	21.4 ± 1.4
10	22.5 ± 2.2	25.2 ± 2.4	14.5 ± 1.6	22.3 ± 5.4	23.8 ± 3.6
50	35.3 ± 7.8	31.3 ± 3.4	20.0 ± 2.3	29.2 ± 6.3	33.3 ± 5.8
100	28.5 ± 6.5	22.8 ± 4.1	20.0 ± 3.1	25.0 ± 7.9	26.8 ± 6.6
500	38.2 ± 8.7	29.0 ± 7.1	23.3 ± 5.1	29.7 ± 8.0	30.0 ± 9.2
1000	47.7 ± 11.5	42.7 ± 10.2	32.8 ± 7.2	39.5 ± 10.1	38.4 ± 13.8

Values are mean ± s.e mean obtained in six subjects, with the exception of the amitriptyline 100 mg condition in which only five subjects were included. (One subject developed orthostatic hypotension accompanied by increased perspiration following the ingestion of amitriptyline (100 mg). Values obtained in the absence of phenylephrine hydrochloride represent 'baseline sweating'.

Table 4 Carbachol- and phenylephrine-evoked sweating: *F*-ratios obtained from analyses of variance.

	Systemic treatment (*d.f. = 4,150*)	*Concentration of intradermally injected drug* (*d.f. = 5,150*)	*Interaction* (*d.f. = 20,150*)
Carbachol	3.42*	22.99**	0.43
Phenylephrine	4.19*	13.20**	0.14

*$P < 0.01$; **$P < 0.001$
For individual comparisons between systemic treatments, see text.

not by binodaline, and that phenylephrine-evoked sweating was reduced both by amitriptyline 100 mg ($P < 0.005$), and binodaline at both dosage levels (50 mg: $P < 0.05$; 100 mg: $P < 0.005$).

Discussion

Binodaline, like placebo, had no significant effect on salivary output, indicating that therapeutically relevant single doses of the drug have little effect on muscarinic receptors. This finding is in agreement with reports from preclinical experiments where no significant antimuscarinic property for the drug could be detected (Adrian *et al.*, 1981; Jahn *et al.*, 1983). In contrast to binodaline, amitriptyline caused a considerable dose-dependent decrease in salivation. This observation is in agreement with previous findings (Ghose *et al.*, 1976; Kopera, 1978; Blackwell *et al.*, 1978; Peck *et al.*, 1979; Szabadi *et al.*, 1980), and is consistent with the well-documented antimuscarinic property of this antidepressant (Atkinson & Ladinsky, 1972; Fjalland *et al.*, 1977; Snyder & Yamamura, 1977).

Binodaline, like placebo, did not affect resting pupil diameter, confirming a previous report by Saletu *et al.* (1980). At first light, this is a surprising finding since binodaline is a potent blocker of noradrenaline uptake (Benfield & Luscombe, 1983), and thus would be expected to potentiate the effect of endogenous noradrenaline, which in turn would result in mydriasis. Indeed, desipramine, a similarly potent blocker of noradrenaline uptake (Møller Nielsen, 1980; Benfield & Luscombe, 1983) is an effective mydriatic agent (Kerr & Szabadi, 1979; Szabadi *et al.*, 1980, 1981; Shur & Checkley, 1982). It has been argued that resting pupil size reflects the balance between pupil dilatation resulting from noradrenaline-uptake blockade and muscarinic receptor blockade on the one hand, and pupil constriction resulting from α-adrenoceptor blockade, on the other hand (Szabadi *et al.*, 1980, 1981). It is possible that, in the case of binodaline, the pupil dilatation resulting from uptake blockade was counteracted by pupil constriction resulting from α-adrenoceptor blockade (see below). Amitriptyline, like binodaline, had no significant effect on resting pupil diameter, confirming earlier reports (Ghose *et al.*, 1974, 1976; Kopera, 1978; Szabadi *et al.*, 1980). The effect of amitriptyline on resting pupil size, however, is variable, and some authors have reported both small increases (Szabadi *et al.*, 1975) and

decreases (Lauber *et al.*, 1976; Kopera, 1978; Peck *et al.*, 1979) in pupil diameter. The lack of a major effect of amitriptyline on resting pupil size is likely to reflect the fact that the mydriasis resulting from muscarinic receptor blockade and noradrenaline-uptake blockade is largely counteracted by the miosis resulting from α-adrenoceptor blockade (Szabadi *et al.*, 1980, 1981). Baseline sweating was not affected by any of the treatments, confirming that it is not a reliable measure of antimuscarinic or adrenolytic properties of systemically taken drugs (Szabadi *et al.*, 1980).

Carbachol-evoked sweating was not affected significantly by binodaline indicating the lack of interaction of this drug with muscarinic receptors. This observation is consistent with the lack of effect of binodaline on salivation (see above), with the lack of an antimuscarinic effect of the drug in isolated tissue preparations (Adrian *et al.*, 1981; Jahn *et al.*, 1983), and with the reported lack of 'anticholinergic' side effects in the clinical situation (Faltus *et al.*, 1981). On the other hand, amitriptyline, in agreement with earlier reports (Szabadi *et al.*, 1980), caused a significant reduction in carbachol-evoked sweating, indicating the potent antimuscarinic effect of this drug (see above).

Phenylephrine-evoked sweating was reduced both by binodaline and amitriptyline. Since phenylephrine is a selective α_1-adrenoceptor stimulant (Ruffolo, 1985), the reduction in phenylephrine-evoked sweating is likely to reflect the blockade of α_1-adrenoceptors by the antidepressants. Indeed, the α_1-adrenoceptor blocking property of amitriptyline is well documented (Scriabine, 1969; Brown *et al.*, 1978; Szabadi *et al.*, 1981). Although there are no data concerning the interaction of binodaline with peripheral α-adrenoceptors, it has been shown in *in vitro* binding experiments that binodaline has some affinity for α_1-adrenoceptors in the cerebral cortex (Roussel Laboratories: unpublished data).

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