RESEARCH PAPERS

THE LOCAL ANAESTHETIC PROPERTIES OF A SERIES OF *N*-SUBSTITUTED *p*-AMINOBENZOIC ACID ESTERS OF TROPINE

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The local anaesthetic properties and toxicities of a series of *N*-substituted *p*-aminobenzoic acid esters of α- and β-tropine were investigated. No consistent differences in local anaesthetic activity were observed between the corresponding α-tropine and β-tropine esters. Substitution at the *p*-amino group by straight chain alkyl radicals having up to four carbon atoms increased both local anaesthetic activity and toxicity. The propyl derivative was as potent as amethocaine but its therapeutic index was lower.

THE effect of replacing one amino hydrogen atom by an alkyl radical on the local anaesthetic properties of diethylaminoethyl *p*-aminobenzoate was reported by Eisleb[1]. Surface anaesthetic activity was found to be little affected when the substituent was methyl but it increased rapidly from ethyl to propyl and then remained fairly constant with straight chains to octyl when it fell rapidly so that the *p*-dodecylaminobenzoic acid ester was almost ineffective.

No systematic examination of the effects of similar substitution in *p*-aminobenzoic acid esters of tropine appears to have been made. The *p*-aminobenzoic acid esters of α-tropine and β-tropine (*pseudo*tropine) are known[2,3] but the only *N*-substituted derivatives for which pharmacological data is available are those with a *p*-*N*-butyl group which are analogous in structure to amethocaine (2-dimethylaminoethyl *p*-butylaminobenzoate). Since both of these tropine esters showed promise as local anaesthetics[3], it seemed worth while examining the properties of other substituted *p*-aminobenzoyl tropeïnes.

METHODS

Surface anaesthesia. The potency of each compound was determined by comparison with amethocaine hydrochloride using a modification of the method described by Chance and Lobstein[4]. A solution of the local anaesthetic was applied to the cornea of a guinea pig for 15 seconds and tests for anaesthesia were carried out at minute intervals for 10 minutes. These tests consisted of touching the cornea with the rounded tip of a fine flexible glass rod and observing whether the corneal blink reflex was elicited. Each compound was evaluated on three guinea pigs which were tested daily for 6 days. Solutions of standard and test compounds dissolved in 0·9 per cent (w/v) NaCl solution at concentrations x, 2x and 4x were applied to the eyes in random order so that at the end of 6 days each solution had been tested on each eye. The mean percentage response for each concentration of drug was plotted against concentration on

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logarithmic probability paper and relative potency estimated from the concentrations giving 50 per cent effects.

Infiltration anaesthesia. The intradermal weal test of Bülbring and Wajda[5] was used. Each compound was usually evaluated on six guinea pigs against procaine hydrochloride. Solutions of standard and test compounds in 0·9 per cent (w/v) NaCl solution at concentrations of x, 2x and 4x were injected intradermally in random order at six sites marked on the back of the animal. Depilation was done on the day before the test with barium sulphide cream after clipping the hair short. The mean response for each concentration of drug was plotted against the logarithm of the concentration and relative potencies were estimated by comparing the concentrations needed to give 50 per cent effects.

Conduction anaesthesia. This was determined in mice weighing between 20 and 30 g. which were tested by pinching the tail with rubber covered

TABLE I

N-SUBSTITUTED *p*-AMINOBENZOIC ACID ESTERS OF TROPINE AND *pseudo*TROPINE

Cpd. No.	m.p. of salt	m.p. or b.p. of ester°C	Found*				Theory			
			C	H	N	Cl	C	H	N	Cl
5	272	200–2/2 mm.	62·1	7·3	9·1	11·3	61·9	7·1	9·1	11·5
6	291–2	205–7/2 mm.	63·4	7·8	8·4	10·9	63·0	7·7	8·6	11·0
7	106–7	—	70·9	7·7	6·8	—	70·8	7·5	6·6	—
8	225–6	206–8/2 mm.	64·0	8·0	8·4	10·4	63·8	8·0	8·3	10·5
9	248–9	112–4	63·7	8·1	8·5	10·3	63·8	8·0	8·3	10·5
10	232–4	190–2/0·5 mm.	63·8	8·2	8·2	10·4	63·8	8·0	8·3	10·5
14	242–3	—	67·6	6·7	7·0	9·1	68·4	7·0	7·2	9·2

* Analyses by Messrs. Weiler and Strauss, Oxford.
Compound 3 contained 10·6 per cent ionisable chlorine (theory 10·7) m.p. 281–2°.

artery forceps. Only those which squeaked on the first or second application of this stimulus were employed in the test. Compounds were dissolved in 0·9 per cent (w/v) NaCl solution and 0·05 ml. of solution was injected subcutaneously bilaterally at the root of the tail. The animals were retested at intervals and the proportion failing to respond to two stimuli recorded.

Duration of local anaesthesia was determined on the rabbit cornea after flooding the eye with drug solution for 30 seconds. Drugs were dissolved in citrate buffer pH 6·0 (Na_2HPO_4 0·2M, citric acid 0·1M). Amethocaine hydrochloride which was used as the standard was applied to one eye and the compound under test to the other. The cornea was then touched every 4 minutes with a fine glass rod and the time for return of the blink reflex noted.

Toxicity tests were carried out in albino mice weighing between 18 and 22 g. Drugs were dissolved in 0·9 per cent (w/v) NaCl solution and 0·5 ml. of solution injected intraperitoneally for each 20 g. weight. The LD50 values with 95 per cent fiducial limits were calculated by the method of Litchfield and Wilcoxon[6].

The mydriatic properties of compounds were estimated by the method of Pulewka modified by Ing, Dawes and Wajda[7]. Atropine sulphate was used as standard and pupil diameters were measured 30 minutes after intraperitoneal injection of a compound.

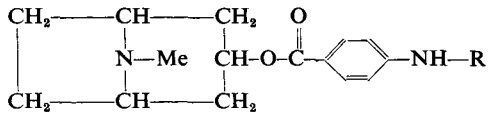
Chemical

p-*Alkylaminobenzoic acids.* These were most conveniently prepared by the action of alkyl bromides on potassium *p*-aminobenzoate[3], with the exception of the *p*-methylamino acid which was obtained in 92 per cent yield by the method of Cosulich and Smith[8].

p-*Alkylaminobenzoyl chlorides.* The hydrochlorides of these were made in 70–90 per cent yields by addition of the acid (60–90 minutes) to thionyl

TABLE II

THE RELATIVE LOCAL ANAESTHETIC POTENCIES AND TOXICITIES OF *N*-SUBSTITUTED *p*-AMINOBENZOIC ACID ESTERS OF TROPINE AND *pseudo*TROPINE



Cpd. No.	C	R	Salt used	Relative potency: Infiltration anaesthesia (guinea pigs)	Relative potency: Surface anaesthesia (guinea pig cornea)	Relative potency: Mydriasis (mice intraperitoneal)	LD50 mg./kg. with P, 0·95 limits (mice intraperitoneal)
1	α	H	Hydrochloride	0·2	0·03	<0·01	33 (30–37)
2	β		Hydrochloride	0·2	—	<0·01	88 (75–104)
3	α	H*	Hydrochloride	0·4	—	—	—
4	α		Acetate	0·2	0·2	<0·01	21·5 (18·7–25·0)
5	α	Me	Hydrochloride	0·3	0·2	<0·01	28 (24–33)
6	α	Et	Hydrochloride	0·7	0·5	—	12·2 (11·4–13·2)
7	α		Phenylacetate	0·8	0·4	0·03	12·1 (11·1–13·2)
8	α	Pr^n	Hydrochloride	1·2	1·0	0·02	11·3 (8·4–15·1)
9	β		Hydrochloride	0·8	0·8	<0·02	12·7 (10·8–15·1)
10	α	Pr^i	Hydrochloride	0·6	0·2	0·01	21 (19–23)
11	α	Bu^n	Hydrochloride	1·0	0·8	—	15·2 (14·0–16·5)
12	β		Hydrochloride	1·0	0·3	<0·01	20 (18–23)
13	α		Acetate	0·6	1·1	<0·01	20 (17–25)
14	α	$-CH_2-C_6H_5$	Hydrochloride	0·2	0·3	<0·01	19 (16–24)
Cinchocaine			Hydrochloride	—	1·3	—	29·5 (25·7–33·9)
Amethocaine			Hydrochloride	1·0	1·0	—	50 (39–65)
Procaine			Hydrochloride	0·1	—	—	220 (188–255)
Atropine			Sulphate	—	—	1·0	—

All values are given in terms of base.
* = Derivative of 2-chloro-4-amino benzoyltropeïne.
C = Conformation of acyloxy group.

chloride (2 ml./g.) stirred at 3–6° (cf. Graf and Langer[9]). After a further 3 hours or after complete solution was achieved the product was precipitated by addition of excess dry ether and was in general sufficiently pure for use.

Esters of the above acids. The amino acid chloride hydrochloride was added to a tropanol hydrochloride (10 per cent excess) in dry chloroform (3 ml./g.). After gentle reflux until evolution of hydrogen chloride had ceased, the salts were extracted with water and the solution basified with ammonium hydroxide. Solvent was removed from the dry ether extract and the residue was either distilled or used directly for preparation of a salt. Yields were 60–80 per cent. Analyses are shown in Table I.

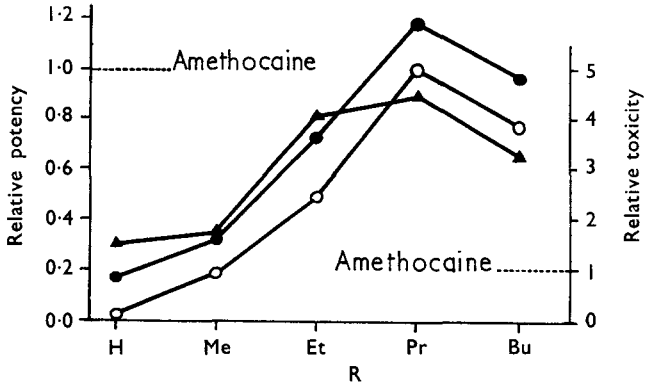


FIG. 1. Local anaesthetic potency and toxicity of α-tropine esters of substituted *p*-aminobenzoic acids *p*-RHN·C_6H_4COOH. All compounds were tested as the hydrochlorides.

●—● Infiltration anaesthesia in guinea pigs.
○—○ Surface anaesthesia in guinea pigs.
▲—▲ Toxicity by I.P. injection in mice.

RESULTS

The results obtained with all compounds in tests for infiltration and surface anaesthetic properties are summarised in Table II. *p*-Aminobenzoyl α-tropeïne was approximately twice as potent as procaine by the intradermal weal test and about one-thirtieth as potent as amethocaine on the guinea pig cornea. Substitution of one hydrogen of its primary amino group by straight chain alkyl groups containing from one to four carbon atoms gave compounds which were both more effective as local anaesthetics and more toxic. These effects are shown in Figure 1. A methyl group augmented activity only slightly but ethyl and propyl groups each gave an increase in potency. Butyl was no more effective than propyl and the results suggest that a peak effect is obtained with the propyl derivative. In this homologous series results obtained for surface anaesthesia paralleled those obtained by infiltration. There was a rough parallelism too between toxicity and local anaesthetic activity.

By intraperitoneal injection in mice the least toxic member of this series was *p*-aminobenzoyl α-tropeïne which had an LD50 of 33 mg./kg.

The corresponding values for the butyl and propyl derivatives were 15·2 mg./kg. and 11·3 mg./kg. respectively. These last two compounds were as effective as amethocaine and cinchocaine in producing local anaesthesia but both were considerably more toxic, the LD50 for cinchocaine being 29·5 mg./kg. and that of amethocaine 50·0 mg./kg.

TABLE III

TOXICITY OF LOCAL ANAESTHETICS BY INTRAVENOUS INJECTION IN MICE

Compound employed	LD50 mg./kg. of base (P 0·95 limits)
Cinchocaine hydrochloride	6·2 (5·5–7·0)
Amethocaine hydrochloride	8·8 (7·9–9·8)
p-Propylaminobenzoyl α-tropeïne hydrochloride	4·6 (4·0–5·2)

TABLE IV

THE RELATIVE DURATION OF ANAESTHESIA PRODUCED BY *p*-PROPYLAMINOBENZOYL α-TROPEÏNE HYDROCHLORIDE AND AMETHOCAINE HYDROCHLORIDE ON THE RABBIT CORNEA

Compound	Concentration mg./ml.	Mean duration of anaesthesia in min. with SD	Proportion of animals anaesthetised
Amethocaine HCl	0·5	22	4/5
	1·0	31 ± 12	5/5
	2·0	44 ± 13	5/5
	4·0	80*	5/5
p-Propylaminobenzoyl α-tropeïne HCl	0·5	6	1/5
	1·0	48 ± 12	5/5
	2·0	77 ± 11	5/5
	4·0	109**	5/5

* Local anaesthesia lasted for over 2 hours in 1/5 rabbits.
** Local anaesthesia lasted for over 2 hours in 4/5 rabbits.

On intravenous injection into albino mice *p*-propylaminobenzoyl α-tropeïne was found to be more toxic than amethocaine and cinchocaine (Table III).

Direct comparison of the duration of anaesthesia on the rabbit cornea after amethocaine hydrochloride and *p*-propylaminobenzoyl α-tropeïne hydrochloride showed that although the threshold concentration was lower for amethocaine, at concentrations giving complete anaesthesia the action of *p*-propylaminobenzoyl α-tropeïne hydrochloride was more prolonged (Table IV).

p-Propylaminobenzoyl α-tropeïne hydrochloride was much less effective than lignocaine hydrochloride in producing anaesthesia in mice (Fig. 2). The peak effect with lignocaine hydrochloride 0·5 per cent was obtained

within 10 minutes of injection and the response to a painful stimulus was abolished in 90 per cent of mice at that time. *p*-Propylaminobenzoyl α-tropeïne hydrochloride produced its maximum effect between 20 and 30 minutes after injection, 0·2 and 0·4 per cent solutions being effective in only 53 and 72 per cent of animals respectively. Both concentrations produced toxic signs (tremors and ataxia) and at the higher dose 2 out of 15 animals died within 20 minutes of injection.

The esters examined showed negligible mydriatic properties in mice after intraperitoneal injection (Table II).

The isopropyl derivative of *p*-aminobenzoyl α-tropeïne was less toxic than the propyl analogue but its local anaesthetic properties were correspondingly reduced. Neither substitution of benzyl at the primary amino

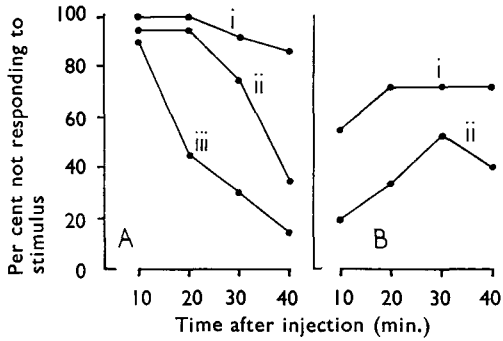


FIG. 2. Conduction anaesthesia in mice. A. Lignocaine hydrochloride. (i) 2 per cent, 15 animals; (ii) 1 per cent, 20 animals; (iii) 0·5 per cent, 20 animals. B. *p*-Propylaminobenzoyltropeïne hydrochloride. (i) 0·4 per cent, 15 animals; (ii) 0·2 per cent, 15 animals. With 0·4 per cent solution two mice died within 20 minutes of injection.

group or chlorine at the *ortho* position in the benzene ring of *p*-aminobenzoyl α-tropeïne was of significance in increasing local anaesthetic activity or reducing toxicity.

In the hope of obtaining less toxic compounds and yet retaining local anaesthetic potency the *p*-aminobenzoic acid ester or β-tropine and its *p*-propyl and *p*-butyl derivatives were prepared since esters of the β isomer have been reported to be less toxic than those of α-tropine[3]. A marked difference in toxicity was observed only in the case of the β-tropine ester of *p*-aminobenzoic acid which was much less toxic than the α form. By the intradermal weal test there were no consistent differences between isomers but in the test for surface anaesthesia the butyl β-tropine ester was considerably less effective than the corresponding α form.

In view of the claim by Rabinovitch and others[3] that acetic or phenylacetic acid esters of tropine were more effective as local anaesthetics than the hydrochlorides, the acetates or phenylacetates of three compounds were tested but were found to have no striking advantages over their hydrochlorides. The local anaesthetic properties of *p*-ethylaminobenzoyl α-tropeïne as the phenylacetate differed only slightly from those of the

hydrochloride. With *p*-butylaminobenzoyl α-tropeïne the potency of the acetate by intradermal injection was less than that of the hydrochloride but on application to the cornea the situation was reversed, the acetate being slightly more effective.

Discussion

Our results confirmed the high activity of *p*-butylaminobenzoyl α-tropeïne reported by Rabinovitch and others[3] and showed that in potency, the local anaesthetic properties of both it and *p*-propylaminobenzoyl α-tropeïne compared favourably with those of amethocaine or cinchocaine when tested by infiltration or application to a mucous surface. However, none of the tropine or *pseudo*tropine esters tested were superior to currently available local anaesthetics. Enhanced local anaesthetic activity was accompanied by increased toxicity and the therapeutic index of the most active compound tested was considerably less than that of amethocaine.

Because the tropine esters were so toxic and it was probable that peak local anaesthetic activity was obtained with the propyl derivative the series was not extended to include compounds in which the length of substituent on the primary amino group was increased beyond butyl.

The results obtained in intradermal weal tests with *p*-aminobenzoyl tropeïne and its *N*-substituted propyl and butyl derivatives did not give any indication that stereoisomerism at the 3 position is of importance in determining the local anaesthetic properties of tropine esters[10,11] and a significant reduction in toxicity by replacing tropine by *pseudo*tropine was only seen in the case of the *p*-aminobenzoic acid ester[3].

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