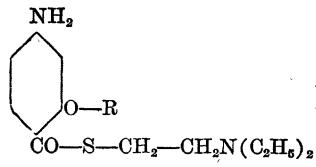
A New Series of Highly Active Local Anesthetics

F. P. Luduena, R. O. Clinton, and S. C. Laskowski

Sterling-Winthrop Research Institute, Rensselaer, New York

Previous reports from this laboratory (*1–3*) have described highly potent local anesthetics obtained by 2-ethoxy or higher 2-alkoxy substitution of the molecule of procaine. Local anesthetic activity, toxicity, and irritancy increased with the length of the alkoxy side chain, but the 2-ethoxy, the 2-*n*-propoxy and the 2-*n*-butoxy analogs were relatively much less irritating than procaine. It has now been found that, by sulfur substitution of the non-carbonyl oxygen in these and related alkoxy derivatives, an additional pronounced increase in activity can be obtained.

The thiolbenzoates used in the present work were prepared (*4*) from 2-alkoxy-4-nitrobenzoic acids and 2-diethylaminoethane thiol by methods analogous to those previously outlined (*5*). Local anesthetic activity was determined by the following methods: intracutaneous wheal in guinea pigs (Bülbring and Wajda's method [*6*]); intraspinal injection in rabbits (method of Bieter *et al.* [*7*]); corneal instillation in rabbits and urethral injection in rabbits (*8*). In all the methods linear dose-response curves were obtained by plotting the average scores or the average duration of anesthesia against the logarithm of the concentration or the concentrations drawn on a logarithmic scale. The activity ratios were determined by the ratios of the threshold anesthetic concentrations, which were estimated from the dose-response curves.



	R	Salt
WIN 3766	*n*-propl	H_3PO_4
WIN 3800	*n*-butyl	HCl
WIN 4510	*n*-hexyl	HCl

The local anesthetic activity and toxicity of these compounds in comparison with procaine, cocaine, tetracaine, and dibucaine are shown in Table 1. All the values are expressed or have been calculated in terms of the bases. Procaine has been taken as unity for activity determined by the intracutaneous wheal method and by intraspinal injection. Topical anesthetic activity was expressed by cocaine ratios, and toxicity was estimated in relation to both procaine and cocaine.

As shown in Table 1, WIN 4510 was the most active and most toxic of the compounds tested. After intraspinal injection in rabbits, a solution of 0.44% procaine (all values in the text are also expressed in terms of the bases) produced anesthesia in 3 of 4 trials for an average of 3.4 min, and a solution of 0.0014% of WIN 4510 produced anesthesia in all 5 rabbits for an average of 8 min.

Even more outstanding was the effect of these new compounds on the cornea and urethra. One of the smallest effective concentrations of WIN 4510, 0.00034%, produced corneal anesthesia in 7 of 8 rabbits for an average of 34 min. The total dose dissolved in approximately 0.5 ml of saline solution placed in contact with the cornea for 1 min was about 1.7 μg (*9*).

The anesthetic effect on the urethra was determined by injecting 0.5 ml of the solution into the urethra of lightly morphinized rabbits. The presence or absence of the urethral reflex (*10*) provoked by electrical stimulation (*8*) was used as a criterion of anesthesia. Each of 4 concentrations of WIN 4510 was tested on 8 or more rabbits. The lowest effective concentration was 0.00045%. Cocaine at 0.45% concentration produced anesthesia for an average duration of 5 min.

The irritancy of these compounds, as measured by the trypan blue test, increased with the length of the alkoxy side chain. It was low in comparison with the anesthetic activity. WIN 3766 had the highest activity/irritancy ratio of a large series of benzoate and thiolbenzoate derivatives tested in this laboratory (*11*).

Other related thiolbenzoates also possess high topical anesthetic activity. Several compounds in this

TABLE 1

ACTIVITY AND TOXICITY OF VARIOUS LOCAL ANESTHETICS

Compound	Local anesthetic activity				Toxicity		
	Procaine ratios		Topical cocaine ratios		Intravenous injection in mice		
	Intracutaneous wheal (guinea pigs)	Intraspinal injection (rabbits)	Corneal (rabbits)	Urethral (rabbits)	$LD_{50} \pm$ S.E. mg/kg	Procaine ratio	Cocaine ratio
Procaine	1	1			52.0 ±1.7	1	
Cocaine			1	1	19.0 ±1.0		1
Tetracaine			8.5		7.3 ±0.3		2.7
Dibucaine			11.0		4.7 ±0.3		4.0
WIN 3766	75	110	53.0	75	0.53 ± 0.02	98	36.0
WIN 3800	75	230	110.0	74	0.53 ± 0.02	98	36.0
WIN 4510	117	330	1000.0	1000	0.32 ± 0.02	160	60.0

series, especially WIN 3800 and WIN 4510, not only possess anesthetic activity considerably greater than that of tetracaine and dibucaine, but the topical anesthetic activity in experimental animals also indicates a greater margin of safety with regard to both irritancy and systemic toxicity.

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Parathyroid and Bone Citrogenase

H. R. Perkins and T. F. Dixon[1]

Institute of Orthopaedics,
Royal National Orthopaedic Hospital,
Brockley Hill, Stanmore, Middlesex, England

It is well known that when the parathyroid glands are removed the serum calcium falls, and, according to Albright and Reifenstein (*1*), the bones tend to become more dense in man, whereas after injection of parathyroid hormone the serum calcium rises and, in rabbits, a reduction of trabeculae of the epiphyses can be shown (*2*). Furthermore, in the experiments of Barnicot (*3*) and Chang (*4*) parathyroid tissue placed in contact with bone *in vivo* caused bone resorption, thus demonstrating a direct effect of the gland upon bone tissue. Therefore, in view of the influence of citrate on the solubility of calcium phosphate precipitated from solutions of physiological ranges of concentrations (*5*) and the presence in bone of enzymes governing the formation and further conversion of citrate (*6*), it seemed of interest to study the citrate content and citrogenase activity of bone in parathyroidectomized and normal rats, and in normal rats injected with parathyroid extract.

All animals were male albino rats and were fed on a stock diet of pellets containing 0.48% P and 0.63% Ca. Parathyroidectomized rats were killed 4–6 weeks after operation. In most cases the serum calcium level was determined immediately before sacrifice, the values obtained on the above Ca and P intakes (between 5 and 6 mg Ca/100 ml) indicating reasonable completeness of extirpation of the glands. Parathyroid hormone[2] was injected intraperitoneally 20 hr before sacrifice. In order to avoid any toxic effects, it was first dialyzed against distilled water until no further phenol reaction was obtained.

The proximal end of the tibia combined with the head and distal end of the femur (in each case comprising the epiphyseal and metaphyseal regions) was examined for citrogenase activity, and in some cases for citrate content, by methods previously described (*6*). The results obtained are given in Table 1.

TABLE 1

BONE CITRIC ACID AND CITROGENASE, AND SERUM CALCIUM IN PARATHYROIDECTOMIZED, NORMAL, AND INJECTED RATS*

Condition	Age in weeks	Bone citrogenase (mg citric acid formed/g tissue/hr)	Bone citric acid (mg/g tissue)	Serum calcium (mg/100 ml)
Normal	7	0.45 (2)		9.7 (2)
	10	0.24 (6)		9.5 (6)
	11	0.19 (7)	4.6 (4)	9.5 (5)
	24	0.09 (2)		—
4–6 weeks after parathyroidectomy	13	0.02 (5)	4.2 (4)	5.5 (3)
	14	0.0 (2)		5.3 (2)
	15	0.02 (3)		5.5 (2)
	18	0.01 (1)		—
4 days after parathyroidectomy	12	0.22 (3)		6.1 (3)
Normal, injected with parathyroid extract (150 u)	10	0.20 (3)		10.2 (3)
4 days after parathyroidectomy, injected with parathyroid extract (300 u)	12	0.21 (2)		12.5 (2)

* Figures in perens indicate the number of animals in the groups.

It can be seen that several weeks after parathyroidectomy the bone citrogenase activity had fallen to a very low level, far lower than that of normal animals of similar age range, whereas in the few determinations made the citrate content of the bones did not differ in the two groups. On the other hand, injection of a massive dose of parathyroid hormone into 10-week-old normals did not lead to any increase of bone citrogenase activity above the normal level for that age. In a few experiments which were performed on animals 4 days after parathyroidectomy, no lowering of the citrogenase activity was observed although the serum calcium had already reached a low level. Administration of parathyroid extract to such animals led to a considerable rise in serum calcium but no change in bone citrogenase activity. The serum cal-

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[2] Eli Lilly, B.P.C. 1934 units.