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Comparative blocking actions of methylated 3-quinuclidinyl and 3-tropinyl residues in cholinergic systems

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STUDIES with 3-tropinyl aryl esters have indicated that *p*-tolylacetates can block responses in cholinergic systems. In particular, the quaternary ester tropine -*p*-tolylacetate methiodide (I) (Fig. 1) has been shown[1] to block contracture of the gastrocnemius-soleus muscles (GS) in the cat, and to block transmission in the superior cervical ganglion preparation (SCG) in the same animal.[2] Since quaternary heterocyclic amines have variable ganglionic blocking activity,[3] it was of interest to synthesize derivatives of I in which the rigidity of the 3-tropinyl nucleus is increased without loss of spatial accessibility to a quaternary bridging nitrogen function, and to observe the cholinergic-blocking activity of compounds with the general structure, heterocycle-$OCOCH_2C_6H_4CH_3$. A logical heterocyclic moiety for this purpose is the 3-quinuclidinyl residue. Accordingly, an analog of I was prepared in which the *p*-tolylacetate ester of 3-quinuclidinol was converted into its methiodide (II) (Fig. 1). The ganglionic and neuromuscular activity of II was tested in the cat's GS and SCG preparations.

Ester II was prepared from the tertiary ester 3-quinuclidinyl-*p*-tolylacetate–HCl (Aldrich Chemical Company) by reacting the free base with methyl iodide. After recrystallization from 50% acetone–ether the product, II, had a constant m.p., 148–149°. Solutions of II were prepared in isotonic saline.

The isometric tetanic tension of the cat's GS preparation elicited by supramaximal electrical stimuli of 0·1-msec duration, applied at the rate of 40 Hz for 1·5 to 2·0 sec to the sciatic nerve, was recorded. Each train of stimuli was followed by a rest period of at least 30 sec. The neuromuscular-blocking effects of II were measured during the infusion of its isotonic 10^{-8} to 10^{-2} M solutions into the popliteal artery, close to the junctional area, at the rate of 1·15 ml/min. Details of the technique were described earlier.[4,5]

The SCG preparation in the anesthetized cat was mounted as previously described,[2,6] and for each evoked response was stimulated electrically via the preganglionic nerve trunk by supramaximal, square wave stimuli of 0·1-msec duration, at 28 Hz for 15 sec. Injections of ester solutions (0·10 ml) in isotonic saline were made into the lingual artery. Contraction/relaxation of the nictitating membrane was followed in time via the output of a membrane-coupled strain gauge and its associated Oscilloriter, model P2CDH.

Dose–response curves for the blocking action of ester II on the cat GS and SCG preparations over the concentration range 10^{-8} to 10^{-2} M in the perfused/injected solutions were compared with previously recorded data[1,2] for I. Dosages were log spaced over the full range, and blockade results at each dose level were recorded in terms of means ± S.D. for three preparations at reference times of ester infusion (GS) or post-injection (SCG). The results obtained with I and II are summarized in Table 1.

The results of Table 1 afford comparison of the relative contributions of the 3-tropinyl (3-T) and 3-quinuclidinyl (3-Q) residues to the neuromuscular and ganglionic blocking effects of I and II respectively. The relative neuromuscular potency of II is about one order higher than that of I. In

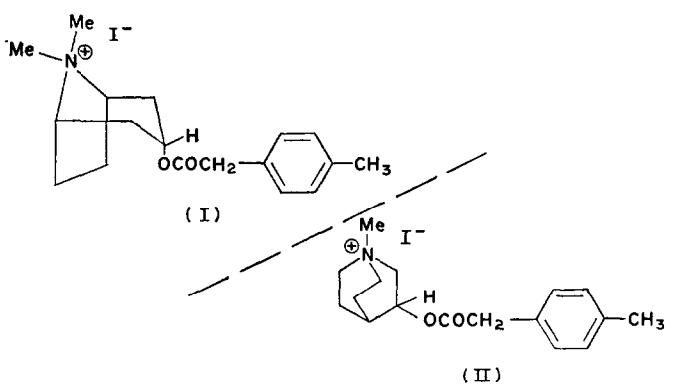

FIG. 1. Structures of tropine-*p*-tolylacetate methiodide (I) and 3-quinuclidinyl-*p*-tolylacetate methiodide (II).

TABLE 1. COMPARISON OF THE POTENCIES OF I AND II IN THE GS AND SCG PREPARATIONS OF THE CAT

Preparation	Ester I*		Ester II	
	Concn (M)	Block (%)	Concn (M)	Block (%)
Gastrocnemius-soleus (GS)†	1×10^{-2}	>50	1×10^{-3}	>50
	5×10^{-3}	Stimulation, followed by 25–50	5×10^{-4}	25–50
	$\leq 1 \times 10^{-4}$	0–10	10^{-4}–10^{-8}	0–10
Superior cervical ganglion (SCG)‡	1×10^{-2}	>50	1×10^{-2}	25–50 (transient)
	1×10^{-3}	10–25	1×10^{-3}	10–25 (transient)
	$\leq 1 \times 10^{-5}$	0	$\leq 1 \times 10^{-5}$	0

* Data from Refs. 1 and 2.
† Duration of infusion $\geq$ 600 sec.
‡ Values at times 5–10 min post-injection of ester aliquots.

contrast the ability of II to block transmission in the SCG is slightly less than that of I. Since transmission in the SCG preparation is in part cholinergic[7] and can be stimulated by the intra-arterial injection of 1×10^{-3} M aliquots of acetylcholine chloride, the reasons for the loss of the relative potency of II in this preparation are obscure.

An attempt to probe this loss of activity was based on a possible difficulty of access of the quaternary II to ganglionic receptor sites, which might be alleviated in a tertiary analog. A test of this possibility was made using the tertiary ester 3-quinuclidinyl-*p*-tolylacetate (III) as the injected ganglionic blocking agent over the test range 10^{-2} to 10^{-8} M, under the same conditions employed to study the effects of II. With III, blockade of moderate strength (25–50 per cent) was noted near an injection level of $0{\cdot}8 \times 10^{-2}$ M, falling to weak blockade (10–25 per cent) near $0{\cdot}8 \times 10^{-3}$ M. Therefore, no improvement in blockade potency of the tertiary ester over its quaternary analog can be established. This result cannot be uniquely interpreted in terms of equivalent penetration ease on the part of III and II, however, since it is possible that the similar ganglionic blockade potency of III and II could reflect a better penetration of the less active tertiary ester III.

Therefore, on the basis of the experimental evidence presently available, it appears that the major effect of a structural change in I leading to increased rigidity is an increased blockade potency at the neuromuscular junction and a slight potency decrease at the superior cervical ganglion. A probable inference from this observation is that the structures of the cholinergic receptors in these tissues may be different.

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REFERENCES

1. S. L. FRIESS, R. C. DURANT, H. L. MARTIN, W. V. HUDAK and H. WEEMS, *Toxic. appl. Pharmac.* **14,** 119 (1969).
2. S. L. FRIESS, W. V. HUDAK and H. WEEMS, *Toxic. appl. Pharmac.* **15,** 540 (1969).
3. K. NADOR, in *Progress in Drug Research* (Ed. E. JUCKER), Vol. 2, pp. 297–416. Interscience, New York (1960).
4. F. G. STANDAERT, *J. gen. Physiol.* **47,** 987 (1964).
5. S. L. FRIESS, R. C. DURANT, H. L. MARTIN, W. V. HUDAK and H. WEEMS, *Toxic. appl. Pharmac.* **13,** 99 (1968).
6. F. G. STANDAERT, S. L. FRIESS and R. O. DOTY, *J. mednl. pharm. Chem.* **1,** 459 (1959).
7. G. B. KOELLE, in *The Pharmacological Basis of Therapeutics* (Eds. L. S. GOODMAN and A. GILMAN), Chapter 21. Macmillan, New York (1965).