Br. J. Pharmac. (1976), **58**, 157–159

ACTION OF SURUGATOXIN ON NICOTINIC RECEPTORS IN THE SUPERIOR CERVICAL GANGLION OF THE RAT

D.A. BROWN & J. GARTHWAITE

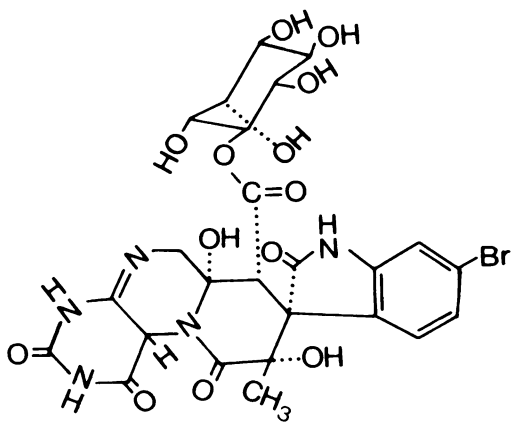
Department of Pharmacology, The School of Pharmacy, University of London, 29/39, Brunswick Square, London, WC1N 1AX

E. HAYASHI & S. YAMADA

Department of Pharmacology, Shizuoka College of Pharmaceutical Sciences, 2-2-1 Oshika, Shizuoka, Japan

Surugatoxin (SGTX, 0.1–2 μM) reversibly depressed orthodromic transmission and antagonized the depolarizing action of carbachol on the isolated superior cervical ganglion of the rat. The apparent dissociation equilibrium constant against carbachol-induced depolarization (measured in the presence of hyoscine) was 58 and 76 nM determined at 0.2 and 2 μM respectively. SGTX (2 μM) did not reduce the depolarizing effects of (±)-muscarine, γ-aminobutyric acid or angiotensin, but did reduce that to 5-hydroxytryptamine. Release of [^{3}H]-acetylcholine following repetitive (10 Hz) preganglionic sympathetic stimulation was maintained in the presence of 2 μM SGTX. It is concluded that SGTX has a high and selective affinity for ganglionic nicotinic receptors.

Introduction Surugatoxin (SGTX) is a toxin extracted from the Japanese ivory mollusc *Babylonia japonica*, with a molecular formula $C_{25}H_{26}N_5O_{13}Br.7H_2O$ (molecular weight 810.5) and molecular structure as follows (Kosuge, Zenda & Ochiai, 1972):



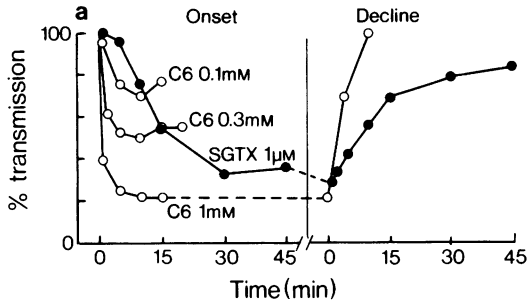
Hayashi & Yamada (1975) found SGTX to be a potent antagonist of nicotinic agonists in the cat superior cervical ganglion and guinea-pig ileum, with little effect on skeletal muscle or smooth muscle receptors, and concluded that poisoning resulted from ganglion-block.

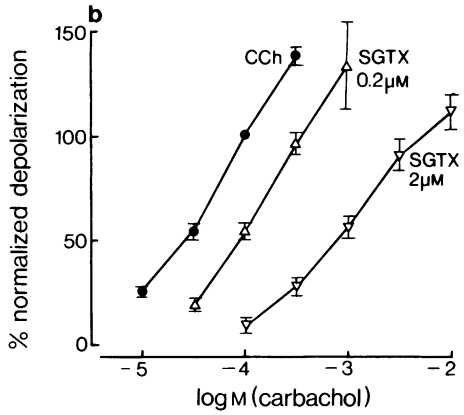
We have recently investigated the action of SGTX on isolated ganglia of the rat and have confirmed its high affinity for nicotinic receptors therein.

Methods Superior cervical ganglia were isolated from rats anaesthetized with urethane and were maintained in Krebs solution at ambient temperature (27–30°C) bubbled with 95% O_2 and 5% CO_2 mixture (Brown, Brownstein & Scholfield, 1972). Depolarizing responses to agonists were recorded potentiometrically by the use of surface electrodes and a superfusion technique (Brown & Marsh, 1975). Ganglionic action potentials elicited by preganglionic nerve stimulation were recorded with a triple-chamber bath (Bowery & Tullett, 1975) and capacitor-coupled amplification (time-constant 10 seconds). In one experiment the triple-chamber bath was also used to measure the release of acetylcholine by preganglionic stimulation, as [^{3}H]-acetylcholine (see Brown, Halliwell, Jones & Quilliam, 1970). The ganglion chamber was perfused for 2 h with 0.8 μM [^{3}H]-choline and the subsequent release of tritium by 4 min periods of 10 Hz preganglionic stimulation measured. [^{3}H]-choline was obtained from the Radiochemical Centre, Amersham. Pure crystalline SGTX was a gift from Professor T. Kosuge.

Results SGTX (1 μM) produced a slowly-developing block of the response to orthodromic transmission (Figure 1a). Though slower in both onset and decline, its effect resembled that of hexamethonium in the following respects: (i) the N-wave (synaptic potential) was strongly depressed; and (ii) block was intensified on raising the stimulus frequency from 1 Hz to 10 Hz.

Dose-depolarization curves for carbachol (measured in the presence of 2.9 μM hyoscine) were





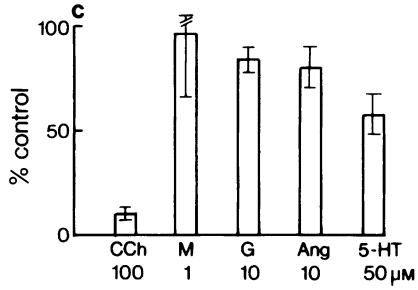


Figure 1 (a) Effects of hexamethonium (C6) and surugatoxin (SGTX) on the amplitude of the ganglionic action potential in response to supramaximal 1 Hz, 1 ms preganglionic nerve stimulation. Ordinates, % of initial amplitude; abscissae, time after adding and washing out the blocking agent. (b) Depolarizations produced by 1 min applications of carbachol at 30 min intervals in the absence and presence of surugatoxin (SGTX). Ordinates: depolarization, expressed as % of that produced by 100 μM carbachol in the absence of SGTX (●). Abscissae: log molar carbachol concentration. Each point is the mean of 4 control experiments or of 3 experiments in SGTX solution (vertical lines show standard errors). (c) Depolarizing responses to standard concentrations of carbachol (100 μM, CCh), (±)-muscarine (M, 1 μM), γ-aminobutyric acid (G, 100 μM), angiotensin (Ang, 10 μM) and 5-hydroxytryptamine (5-HT, 50 μM) in the presence of 2 μM SGTX, expressed as % of control responses (means and standard errors of 4 experiments).

shifted in an approximately parallel manner by SGTX (Figure 1b). Apparent dissociation constants measured at equilibrium block for SGTX were 58 nM (measured from the shift produced by 0.2 μM SGTX) and 76 nM (measured at 2 μM). The apparent dissociation constant for hexamethonium against carbachol was 7.6 μM (at 10 μM hexamethonium) and 19.7 μM at 100 μM hexamethonium. SGTX did not depolarize the ganglion.

At a concentration of 2 μM, SGTX did not significantly reduce the depolarizations produced by γ-aminobutyric acid (GABA, 100 μM), (±)-muscarine (1 μM), or angiotensin (10 μM), but appeared to reduce the response to 5-hydroxytryptamine (50 μM) by some 44% (Figure 1c).

In one test, SGTX (2 μM) did not reduce the neurally-evoked release of tritium from a ganglion previously labelled with [^{3}H]-choline; in the same experiment, release was reversibly suppressed by a 20 mM[Mg^{2+}], Ca^{2+}-free solution.

Discussion The present observations confirm the high affinity of SGTX for ganglionic nicotinic receptors deduced by Hayashi & Yamada (1975) from tests on the guinea-pig ileum: its apparent affinity constant is at least two orders of magnitude greater than that of hexamethonium. The slow decline of ganglion block would accord with this. Slow onset is unlikely to be due to restricted diffusion *per se*, since SGTX is not a large molecule; rather, it may reflect a buffering effect of receptor-binding on the interstitial concentration (as with tetrodotoxin: cf. Colquhoun, Henderson & Ritchie, 1972).

There was some suggestion of antagonism to 5-hydroxytryptamine; though requiring further quantitation, this also accords with the observation of Hayashi & Yamada (1975) on the guinea-pig ileum. Other ganglionic receptors for muscarine, GABA and angiotensin appear resistant to SGTX. This, coupled with an inability to reduce acetylcholine release, suggests that SGTX is a fairly specific 'competitive' nicotinic ganglion-blocking agent.

SGTX is of appreciable interest as a 'neurotoxin' with a selective affinity for ganglionic nicotinic receptors. Its uniqueness in this respect is emphasized by the fact that the polypeptide snake venoms so

effective at motor end-plates are generally quite inactive at ganglia (Magazanik, Ivanov & Lukomskaya, 1974; Brown, D.A., unpublished observations). The action of SGTX highlights the distinction between these two types of nicotinic receptor.

We are grateful to Professor T. Kosuge (Shizuoka College of Pharmaceutical Sciences, Shizuoka, Japan) for the gift of surugatoxin, to Dr N.G, Bowery for undertaking the acetylcholine-release experiment and to S. Marsh for his assistance. The investigation was aided by a grant from the American Medical Association Education and Research Foundation to D.A.B.

References

BOWERY, N.G. & TULETT, D. (1975). A technique for the simultaneous measurement of effluent radioactivity and electrical responses in the rat superior cervical ganglion and its pre- and postganglionic trunks *in vitro*. *J. Physiol., Lond.*, **246**, 20–21P.

BROWN, D.A., BROWNSTEIN, M.J. & SCHOLFIELD, C.N. (1972). Origin of the after-hyperpolarization that follows removal of depolarizing agents from the isolated superior cervical ganglion of the rat. *Br. J. Pharmac.*, **44**, 651–671.

BROWN, D.A., HALLIWELL, J.V., JONES, K.B. & QUILLIAM, J.P. (1970). Evidence against a presynaptic action of acetylcholine during ganglionic transmission. *Nature New Biol., Lond.*, **266**, 958–959.

BROWN, D.A. & MARSH, S. (1975). A very simple method for recording ganglion depolarization. *J. Physiol., Lond.*, **246**, 24–26P.

COLQUHOUN, D., HENDERSON, R. & RITCHIE, J.M. (1972). The binding of labelled tetrodotoxin to non-myelinated nerve fibres. *J. Physiol., Lond.*, **227**, 95–126.

HAYASHI, E. & YAMADA, S. (1975). Pharmacological studies on surugatoxin, the toxic principle from Japanese Ivory mollusc (*Babylonia japonica*). *Br. J. Pharmac.*, **53**, 207–215.

KOSUGE, T., ZENDA, H. & OCHIAI, A. (1972). Isolation and structure determination of a new marine toxin, surugatoxin, from the Japanese Ivory shell, *Babylonia japonica*. *Tetrahedron Letters*, **25**, 2545–2548.

MAGAZANIK, L.G., IVANOV, A.Ya. & LUKOMSKAYA, N.Ya. (1974). The effect of snake venom polypeptides on cholinoreceptors in isolated rabbit sympathetic ganglia. *Neurofiziol.*, **6**, 652–656. [In Russian.]

(Received May 5, 1976.)