INHIBITORY ACTION OF 2-(1,2-BENZISOXAZOL-3-YL)-3-[2-(2-PIPERIDINOETHOXY)PHENYL]ACRYLONITRILE (SX-284) ON ACETYLCHOLINE RELEASE FROM VAGUS NERVE IN GUINEA PIG ILEUM

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Newly synthesized compound, 2-(1,2-benzisoxazol-3-yl)-3-[2-(2-piperidinoethoxy)phenyl]acrylonitrile (SX-284) inhibited electrically evoked acetylcholine release from the stores in the myenteric plexus-longitudinal strips of guinea pig ileum which was labelled with [^3H]-choline but not the evoked release of tracer from rat vas deferens after labelling with [^3H]-norepinephrine. These results support our previous findings that SX-284 specifically inhibit acetylcholine release from the vagus nerve in the gastrointestinal canal.

Keywords — SX-284; inhibitor of acetylcholine release; vagus nerve; guinea pig ileum; antispasmodic drug

It was previously reported that a newly synthesized drug, 2-(1,2-benzisoxazol-3-yl)-3-[2-(2-piperidinoethoxy)-phenyl]acrylonitrile (SX-284) specifically inhibited the acetylcholine release from the vagus nerve in the gastrointestinal tract.¹⁾ In order to support our previous findings,¹⁾ we studied the effect of SX-284 on the evoked acetylcholine release from the stores in the guinea pig ileum, which was labelled with [^3H]-choline.

Male guinea pigs, weighing 300–400 g, were killed by a blow on the head and myenteric plexus-longitudinal muscle strips were isolated from the middle ileum.²⁾ The muscle preparations were suspended in a 3 ml organ bath in Krebs solution (pH 7.4) kept at 37°C and gassed with carbogen under a tension of 0.5 g. Krebs solution used had the following composition (mM: NaCl 118, KCl 4.7, KH_2PO_4 0.6, NaHCO_3 25, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 2.5, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.3 and glucose 11). Estimation of acetylcholine concentration released was carried out by the method of Kill-

inger *et al.*³⁾ The tissues were allowed to equilibrate for 45 min and incubated for 90 min in 2.6 ml of Krebs solution containing 10^{-5} Ci/ml (a total concentration of labelled and unlabelled choline: 10^{-7}M) of [^3H]-choline (specific activity; 80 Ci/mmol). During the 90 min incubation with [^3H]-choline, field stimulation was carried out by passing a rectangular pulse of 0.3 ms duration, supramaximal voltage and a frequency of 0.1 Hz between two platinum electrodes that were positioned in parallel to the strips.¹⁾ Twitch responses of the tissue to electrical stimulation was recorded. After the 90 min incubation, the tissue was washed with Krebs solution at a flow rate of 6 ml/min for 40 min. Then the tissue was constantly superfused with Krebs solution in the 3 ml organ bath at a rate of 2.5 ml/min. The composition of Krebs solution was the same as that described above, except that it contained hemicholinium-3 (10^{-5}M) in order to prevent a reuptake of choline; the bath was kept at 37°C. In order to measure an increased efflux of tracer

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evoked by electrical stimulation a half ml of the perfusate collected for 1 ml was added to 10 ml of a toluene-scintillator which contained triton 200 ml, toluene 800 ml, 2,5-diphenyl-oxazole (PPO) 5 g, 1,4-bis (5-phenyl-oxazolyl)benzene (POPOP) 0.1 g in a litre and radioactivity was measured by a liquid scintillation spectrometer. The tissue was electrically stimulated for 1 min at 10, 50 and 90 min after the 40 min washing with Krebs solution. In the control experiments

where test drugs were not used, the efflux of tritium was found to be declined from the second to the third stimulation: the observed values at the second and third stimulations were 70.1 ± 8.0 and $58.4 \pm 3.5\%$ (mean $\pm$ S.E. of 8 experiments) of that (100%) at the first stimulation. In a series of the test-experiments, the first stimulation was applied in the absence of the test drug and then the second and third stimulations in the presence of the test drug. Two con-

FIG. 1. *Inhibitory Actions of SX-284 and Tetrodotoxin on the Efflux of Tracer from the Myenteric Plexus-longitudinal Muscle and Its Twitch Response evoked by Electrical stimulation (Left) and Relationship between the Efflux of Tracer from Preparation and Its Twitch Response in the Presence of SX-284 (Right)*

[Left] Ordinate is expressed as % of the response in the absence of drug and abscissa is concentration (g/ml) of SX-284 or tetrodotoxin. $\circ$: efflux of tracer in the presence of SX-284, $\bullet$: twitch response in the presence of SX-284, $\triangle$: efflux of tracer in the presence of tetrodotoxin and $\blacktriangle$: twitch response in the presence of tetrodotoxin. Each point is presented as mean with S.E. (bar) of 5 experiments.

[Right] The right figure was replotted from the left. Ordinate: Efflux of tracer (%) and abscissa: mechanical response (%). Coefficient of correlation (r) was 0.660 and significant at $p < 0.05$.

TABLE I. *Effects of SX-284 and Tetrodotoxin on Efflux of Tracer from the Rat Vas Deferens and Its Twitch Response*

	Efflux of tracer	Twitch response
SX-284 (10^{-6} g/ml)	116.7 ± 23.5	95.5 ± 11.3
Tetrodotoxin (10^{-7} g/ml)	54.0 ± 5.7	63.0 ± 5.9

Each value is expressed as % of the value in the absence of drug and as a mean $\pm$ S.E. of 4 experiments.

centrations were chosen at random from those indicated in Fig. 1 and applied to the preparations. The effluxes of tritium at the second and third stimulations in the presence of the drug were expressed as percents of that at the first stimulation in the absence of the drug. Effects of test drugs were expressed as ratios of the percents in the test-experiments against the corresponding percents in the control experiments.

In some experiments, effects of drugs on release of norepinephrine from the rat vas deferens were tested according to Hedqvist and von Euler.⁴⁾ Male rats, weighing 200–250 g, were killed by the blow on the head and vas deferens was removed. The isolated vas deferens was set in the same apparatus used in the experiments with the myenteric plexus-longitudinal muscle strips. In this experiments 3×10^{-8} Ci/ml (a total concentration of labelled and unlabelled norepinephrine: 5×10^{-9} M) of [³H]-norepinephrine (47 Ci/mmol) was used instead of [³H]-choline. The perfusate contained 0.1 mM ascorbic acid in order to prevent oxidation of norepinephrine. Vas deferens was transmurally stimulated with a rectangular pulse of 1 ms duration, supra-maximal voltage and a frequency of 1 Hz for 30 s.¹⁾ The responses to electrical stimulations was simultaneously recorded isometrically. The experiments were carried out on the same schedule as described above.

In the myenteric plexus-longitudinal muscle preparation of guinea pig ileum twitch responses and efflux of tracer evoked by electrical stimula-

tion inhibited by SX-284 was dose-dependent. Both responses to stimulation were inhibited by tetrodotoxin (10^{-7} g/ml). A significant correlation between inhibitions of twitch responses and of effluxes of tracer was found (Fig. 1), suggesting that inhibitory action of SX-284 on the twitch responses was due to inhibition of acetylcholine release from the vagus nerves.

The evoked efflux of tracer from the rat vas deferens was inhibited by tetrodotoxin (10^{-7} g/ml) but not by SX-284 (10^{-6} g/ml), which did not influence the mechanical response of vas deferens to electrical stimulation (Table I).

The present results support our previous findings that SX-284 specifically inhibits the acetylcholine release from the parasympathetic nerves.

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