Short Communications

POTENTIATION OF RESPONSE OF FROG RECTUS ABDOMINIS MUSCLE TO ACETYLCHOLINE BY ISATIN*

By

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Torda and Wolf (4) and Izquierdo and Stoppani (2, 3) reported that indole, skatole and L-tryptophane increase the response of skeletal muscle to accetylcholine and potassium.

Since isatin (indoline, 2-3 dione) is an indole derivative it was considered of interest to study its effect on frog rectus abdominis muscle.

MATERIALS AND METHODS

The frog rectus abdominis muscle preparation was set up according to the method of Burn (1). Dose response curves of acetylcholine were constructed using (i) plain frog Ringer solution and (ii) frog Ringer solution to which appropriate amount of isatin had been added. The following sequence was used. The response to a given concentration of acetylcholine was first established. The period of contact was limited to 90 sec at the end of which the drum was stopped and fluid in the bath was changed. After the muscle had relaxed, the plain frog Ringer solution was replaced by frog Ringer solution containing different concentrations of isatin. The muscle was then allowed to equilibrate in isatin Ringer solution for two min; thereafter, the same concentration of acetylcholine was added to the bath and the contraction recorded for 90 sec. The concentration of Ach was increased in a logarithmic fashion until the maximum response was obtained.

The degree of potentiation was calculated as the ratio of ED_{50} of acetylcholine (Ach) obtained in plain frog Ringer to the ED_{50} of Ach obtained in isatin containing frog Ringer.

$$\text{Potentiation factor (PF)} = \frac{ED_{50} \text{ of Ach in plain Ringer}}{ED_{50} \text{ of Ach in isatin Ringer}}$$

All solutions of isatin and acetylcholine were prepared just before the start of experiment. Ach was used as the chloride salt and the concentrations refer to the salt.

RESULTS AND DISCUSSION

Isatin in the dose used had no effect on the muscle. Isatin potentiated response to Ach. The results are shown in Fig. 1. The ED_{50} values of Ach as computed from the Fig. were

* Received 2-7-1970

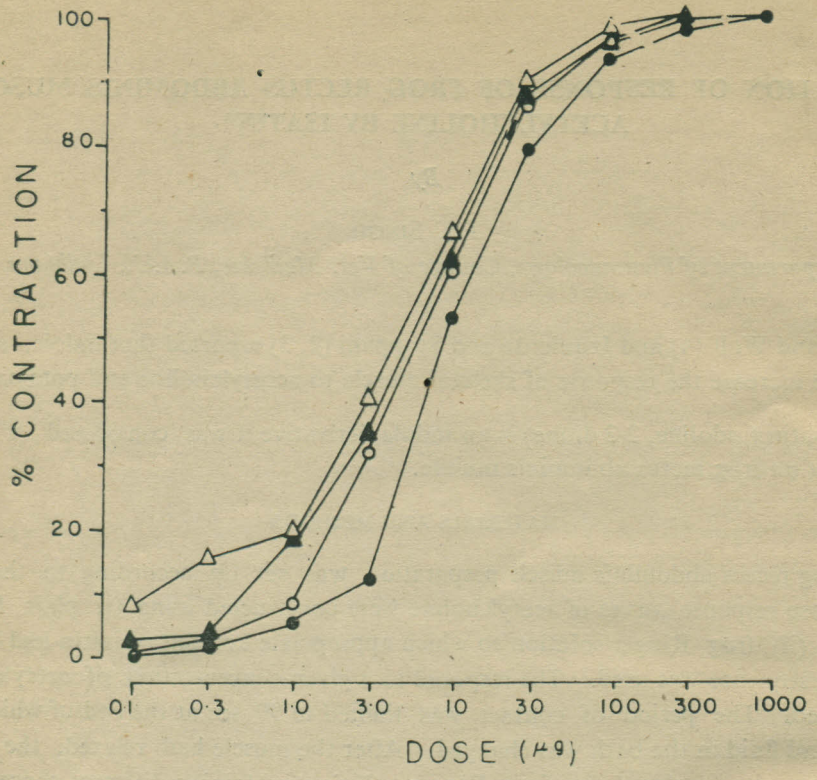

Fig. 1

Isolated frog rectus abdominis muscle. Dose response curves of acetylcholine in plain and isatin containing frog Ringeer. Ordinate; per cent of maximal contraction; abscissa concentration of acetylcholine per ml of frog Ringer solution in bath. Acetylcholine control (●———●); in the presence of isatin 3×10^{-5} (0———0). *isatin* 1×10^{-4} (▲——▲); *and isatin* 2×10^{-4} (△——△). *Each point represents the mean of five observations.*

6.4, μg, 5.7 μg and 4.7 μg, respectively in the presence of isatin 3×10^{-5}, 1×10^{-4} and 2×10^{-4} compared to control value of 9.2 μg. Potentiation factors in the presence of isatin 2×10^{-4}, 1×10^{-5} and 3×10^{-5} were 1.88 ± 0.11*, 1.79 ± 0.12 and 1.42 ± 0.01 respectively. Isatin concentrations higher than 2×10^{-4} could not be used because of low solubility of the drug. The data were analysed by analysis of variance. There was significant difference in the contraction produced by acetylcholine in the presence of isatin as compared to control ($P \leqslant 0.01$).

To elucidate the mechanism of this potentiation, anticholinesterase effect of isatin was studied as follows:—

Four test tubes were prepared as indicated in Table I and were incubated at 37°C for 30 min. At the end of this period, 1.0 *ml* aliquots were withdrawn from each tube and assayed for the presence of acetylcholine on the dog blood pressure.

* Standard deviation.

TABLE I

Plan of testing anticholinesterase activity (see text for details).

Drug	*Tubes*			
	I	*II*	*III*	*IV*
Isatin (1 *mg/ml*)	—	—	1.0 *ml*	—
Plasma	—	0.5 *ml*	0.5 *ml*	0.5 *ml*
Neostigmine (1 *mg/ml*)	—	—	—	0.1 *ml*
Ach (100 *mg /ml*)	1.0	1.0 *ml*	1.0 *ml*	1.0 *ml*
Phosphate buffer pH 7.4	4.0	3.5 *ml*	2.5 *ml*	3.4 *ml*

Intravenous injection of the aliquots obtained from tubes I and IV produced a distinct depressor response. No activity was present in the aliquots from tubes II and III, indicating that isatin present in tube III did not protect acetylcholine from hydrolysis by cholinesterase.

On the basis of these experiments it is reasonable to conclude that isatin does not possess an anticholinesterase activity.

SUMMARY

Isatin (indoline, 2-3 dione) *per se* had no effect upon frog rectus abdominis muscle, but in concentrations of 3×10^{-5}, 1×10^{-4} and 2×10^{-4} w/v, it potentiated the effect of acetylcholine. The potentiation was dose dependent.

REFERENCES

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