J. Pharm. Pharmac., 1977, 29, 542–545 Received February 7, 1977

Influence of pyridylisatogen tosylate on contractions produced by ATP and by purinergic stimulation in the terminal ileum of the guinea-pig

T. KAŽIĆ* AND D. MILOSAVLJEVIĆ†

Department of Pharmacology, Medical Faculty, 11000 Beograd, P.O. B 662 and† Department of Pharmacology, Medical Faculty, Priština, Yugoslavia

2-2′Pyridylisatogen tosylate (PIT), which antagonizes the inhibitory action of ATP in the taenia caeci, did not antagonize the excitatory effects of exogenous ATP and of purinergic stimulation of the terminal guinea-pig ileum. PIT (0·5–2·5 μM) potentiated the ATP-induced contractions and also the contractions produced by potassium chloride, though the potentiation could not be related to the dose in every experiment. The responses to noradrenaline, adrenaline and histamine were slightly inhibited. PIT also potentiated the contractions produced by electrical stimulation of intramural purinergic nerves when either an alternate or uniform stimulation pattern was used. The present results in which the preparation is contracted by ATP are opposite to those obtained with PIT on the taenia caeci, which is relaxed by ATP. This raises a question of duality or plurality of receptors for ATP.

The lack of specific receptor antagonists for adenosine triphosphate (ATP) gives rise to difficulties in defining the selective actions of this nucleotide and related substances. The antagonists used until recently (phentolamine, tolazoline, yohimbine, quinidine, etc.) have been employed in high concentrations that are known to exert diverse pharmacological actions (Boyd, Chang & Rand, 1960; Goodman & Gilman, 1970). In the search for a selective ATP antagonist, 2-2′pyridylisatogen tosylate (PIT) has been shown to selectively antagonize the inhibitory action of ATP on the isolated taenia caeci (Hooper, Spedding & others, 1974; Spedding, Sweetman & Weetman, 1975).

In the terminal ileum of the guinea-pig, which differs in its pharmacology from other regions of the gastrointestinal tract (Munro, 1951; Daniel, 1973), ATP produces a contraction in the presence of atropine while electrical stimulation at 3 Hz gives rise to activation of purinergic mechanisms (Kažić, 1973, 1975). We have extended an investigation into this aspect with particular attention to the possible interaction of PIT with excitatory actions of ATP and of purinergic stimulation.

MATERIALS AND METHODS

Terminal segments of the ileum were dissected from stunned and bled guinea-pigs, 300–600 g. 2–4 cm long pieces taken from a distance 2–3 cm proximal to the ielocaecal valve were suspended in an organ bath of 10 ml volume and left to equilibrate for at least 30 min. They were bathed in Tyrode solution bubbled with oxygen and kept at 36°. Contractions were recorded isotonically.

Electrical stimulation was with coaxially placed electrodes according to Paton (1957), with trains of pulses delivered from a Grass S8 electronic stimulator. The train duration was 1 s for a frequency of 30 Hz and 2 s for a frequency of 3 Hz. The duration of pulses varied with the sensitivity of preparations from 0·2–0·4 ms. Pulse strengths of 10–80 V were used to produce submaximal contractions. Trains of pulses were spaced by 100 s intervals.

Substances used were: adenosine triphosphate dipotassium salt (ATP), atropine sulphate, noradrenaline bitartrate, adrenaline hydrochloride, histamine dihyrochloride, potassium chloride and 2-2′pyridylisatogen tosylate.

RESULTS

Effect of PIT on drug-induced contractions

In the presence of atropine, 3 μM, PIT 0·5–2·5 μM potentiated the contractile response to ATP and potassium chloride whereas the contractions produced by noradrenaline were inhibited (Fig. 1). With 0·5 and 1 μM PIT, the differences between the responses to ATP and noradrenaline were significant ($P < 0·01$ and $P < 0·025$, respectively). The responses to potassium, however, were significantly different from those to noradrenaline only with

* Correspondence.

1 μM PIT ($P < 0.01$). Contrary to this, with 2·5 μM PIT there was no significant difference between the responses to either of the tested substances.

The contractions to adrenaline and histamine were also slightly affected by 2 μM PIT. Inhibition of 14 ± 8 and 10 ± 7% respectively, from the control contraction was observed (n = 8). The

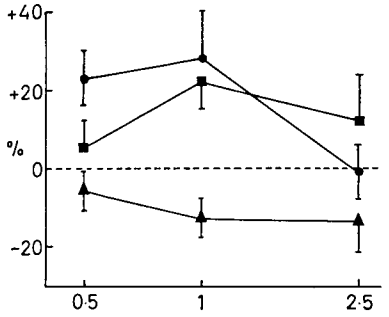

FIG. 1. Effect of PIT on drug-induced contractions of the terminal ileum.—Atropine sulphate 3 μM was present in the bathing fluid. The results are given as means ± s.e.m. from groups of 6 experiments. The values represent change of the corresponding control contractions (%). ■—potassium chloride (13 mM), ●—adenosine triphosphate (0·2 mM). ▲—noradrenaline (1·2 μM). Abscissa—log concentration of PIT (μM).

inhibitory action of PIT was seen in only one half of the experiment, and no change was found in the others. In these experiments PIT was left in contact with the preparations for 60 s and the agonists (ATP, potassium, noradrenaline, adrenaline or histamine) were added while it was in the bath.

Effect of PIT on contractions produced by electrical stimulation

Alternate stimulation. In the presence of atropine 3 μM, electrical stimulation was performed by alternating trains of pulses at 3 and 30 Hz. Electrical stimulation at 3 Hz activates purinergic mechanisms whereas that at 30 Hz elicits contractile responses of adrenergic origin (Kažić, 1975). We found PIT 1 μM to potentiate the responses to purinergic stimulation while the responses to adrenergic stimulation were inhibited (Fig. 2). The time course and the summarized results of this series are presented in Table 1 A. The potentiation of the responses to purinergic stimulation developed promptly but rapidly wore off. Contrary to this, inhibition of the responses to adrenergic stimulation took more time to develop but lasted longer.

Uniform stimulation. In experiments in which the preparations were stimulated uniformly by trains

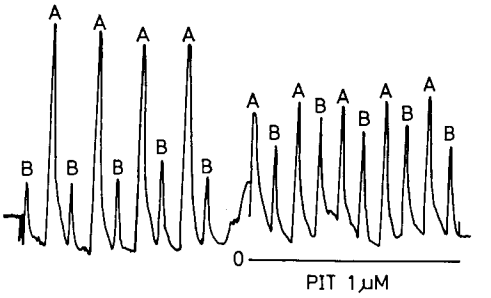

FIG. 2. Effect of PIT on contractile responses of the terminal ileum to electrical stimulation.—Atropine sulphate 3 μM was present in the bathing fluid. A = responses to stimulation at 30 Hz. B = responses to stimulation at 3 Hz. The responses are spaced by 100 s intervals. At the circle, PIT (1 μM) was added into the bath and the bar indicates its continuous contact with the preparation.

of pulses at 3 or 30 Hz, PIT (0·5–2·5 μM) potentiated both types of responses. However, the difference in potentiation of the two types of responses was significant at any given concentration. The responses to purinergic stimulation were in all instances potentiated more than the responses to adrenergic stimulation (Table 1 B).

DISCUSSION

The present experiments provide evidence that the contractions of the terminal ileum to ATP and to electrical stimulation of purinergic intramural nerves are not inhibited by PIT—the substance which has

Table 1. *Effect of PIT on contractions produced by electrical stimulation of the terminal ileum.* Atropine sulphate 3 μM was present throughout in the bath. The results are given as the means ± s.e.m. for groups of 10 (A) and 6 experiments (B). The values represent changes, in percentages, of the control contractile responses to electrical stimulation at either 3 or 30 Hz: + indicates potentiation, − indicates inhibition of the control contractions.

A. Alternate stimulation	PIT 0·5 μM		
	Time course of the effects (min)		
	1–3	3–5	5–7
3 Hz - purinergic	+83 ± 16[a]	+41 ± 16[a]	+20 ± 14[a]
30 Hz - adrenergic	−10 ± 15	−27 ± 9	−32 ± 6
B. Uniform stimulation	PIT μM		
	0·5	1	2·5
3 Hz - purinergic	+35 ± 5[b]	+57 ± 7[b]	+73 ± 13[b]
30 Hz - adrenergic	+ 5 ± 7	+ 16 ± 4	+ 34 ± 6

[a] Responses significantly different from those to 30 Hz stimulation, $P < 0.01$.
[b] Responses significantly different from those to 30 Hz stimulation, $P < 0.05$.

been recently shown to selectively antagonize the inhibitory action of ATP on the taenia caeci (Spedding & others, 1975). In the terminal ileum preparation, ATP and electrical stimulation at 3 Hz (purinergic stimulation) produce excitatory responses in the presence of atropine (Kažić, 1973, 1975). Contrary to the inhibition observed in the taenia caeci, a potentiation by PIT was observed in the present study on the terminal ileum. The contraction produced by purinergic stimulation was consistently potentiated in a dose-dependent manner within the concentration range used. In the study, PIT was used at concentrations (from 0·5–2·5 μM) far lower than those used by Spedding & others (1975). We used these lower concentrations when we found that PIT at concentrations over 10 μM produced an irreversible blockade of contractility of the preparation. Such a non-specific action of PIT presumably stems from its capability of forming covalent bonds with some amino acids in the cell membrane, including areas containing receptors for ATP and other smooth muscle contracting substances (Hooper & Robertson, 1971). We therefore assumed it to be more justifiable pharmacologically to work with concentrations of PIT that produced reversible change in the reactivity of the ileum. With much higher concentrations of PIT, Spedding & others (1975) reported that in the early stages the antagonism of ATP action was competitive.

The congruity of our results obtained with exogenous ATP and with purinergic stimulation lend further support to the purinergic nerve hypothesis (Burnstock, 1972). The present results emphasize that the mechanisms by which exogenous ATP and purinergic stimulation contract the ileum are similar or identical because they are equally facilitated by PIT. The ATP-induced contraction of the terminal ileum has already been shown to be potentiated in the absence of magnesium (Kažić, 1973). In addition to this, nucleotides are believed to contract the smooth muscle by virtue of their ability to complex magnesium in the cell membrane, thereby favouring entry of calcium (Daniel & Irwin, 1964). Therefore, the potentiation by PIT of the excitatory actions of ATP and purinergic stimulation could be accounted for by its ability to combine with magnesium ions, thus facilitating the action of ATP administered exogenously or released from the intramural purinergic nerves by electrical stimulation.

The apparent discrepancy between our results and those of Hooper & others (1974) and Spedding & others (1975) might arise from several sources: different concentrations of PIT were used, the experimental pattern was different and the types of ATP actions examined were opposite. Nevertheless, they underline that there might be more than one type of ATP receptor in the guinea-pig intestine. One of them, situated in the taenia caeci, would mediate inhibition and is blocked by relatively high concentrations of PIT, the other, located in the terminal ileum, would mediate excitation and is activated by PIT.

The potentiating action of PIT on contractions produced by electrical stimulation is unlikely to be specific for purinergic nerve mediated responses, as may be concluded from Fig. 2, in which alternate stimulation was used, because adrenergic responses were also found to be potentiated when a uniform type of stimulation was used (Table 1 B). It should be stressed, however, that the potentiation of purinergic responses was always significantly greater. Thus, it is possible that during alternate stimulation the magnitude of the responses might be interrelated, and that an increased purinergic activation could concommitantly depress the responses to adrenergic stimulation by neurohumoral means. This proposal is also based on our previous finding that ATP is capable of inhibiting the responses to adrenergic stimulation (30 Hz) while leaving those to purinergic stimulation (3 Hz) unchanged (Kažić, 1974).

The results with noradrenaline and other drugs acting directly on the smooth muscle and also those with alternate stimulation suggest that PIT might have a weak anti-adrenergic action, presumably as a selective membrane stabilizer. The experiments with the uniformly adrenergic stimulation, the responses to which were enhanced by PIT, showed that PIT did not interfere with the functioning of the adrenergic neurons.

Acknowledgements

The authors are indebted to M. Spedding for the generous supply of PIT. This research was supported by a grant from ZMNU of SR Serbia.

REFERENCES

BOYD, H., CHANG, V. & RAND, M. J. (1960). *Br. J. Pharmac. Chemother.*, **15**, 525–531.
BURNSTOCK, G. (1972). *Pharmac. Rev.*, **24**, 509–581.

DANIEL, E. E. (1973). In: *Pharmacology of Gastrointestinal Tract Motility and Secretion*, Vol. II, pp. 457–545, Editor: Holton, P. Oxford: Pergamon Press.

DANIEL, E. E. & IRWIN, J. (1964). *Can. J. Physiol. Pharmac.*, **43**, 89–136.

GOODMAN, L. S. & GILMAN, A. (1970). *The Pharmacological Basis of Therapeutics*, 4th edn, pp. 549–584. London: Collier-Mcmillan.

HOOPER, M. & ROBERTSON, J. W. (1971). *Tetrahedron Letters*, 2139–2140.

HOOPER, M., SPEDDING, M., SWEETMAN, A. J. & WEETMAN, D. F. (1974). *Br. J. Pharmac.*, **50**, 458–459.

KAŽIĆ, T. (1973). *Iugoslav. Physiol. Pharmac. Acta*, **9**, 391–398.

KAŽIĆ, T. (1974). *Ibid.*, **10**, 315–322.

KAŽIĆ, T. (1975). *Ibid.*, **11**, 231–240.

MUNRO, A. F. (1951). *J. Physiol., Lond.*, **112**, 84–94.

PATON, W. D. M. (1957). *Br. J. Pharmac. Chemother.*, **12**, 119–127.

SPEDDING, M., SWEETMAN, A. J. & WEETMAN, D. F. (1975). *Br. J. Pharmac.*, **53**, 575–583.