Br. J. Pharmac. (1974), **50**, 609-611

SOME EFFECTS OF DIPYRIDAMOLE, HEXOBENDINE AND LIDOFLAZINE ON INHIBITORY PROCESSES IN RABBIT DUODENUM

MARILYN E. HULME & A.H. WESTON[1]

Department of Pharmacology, Materia Medica & Therapeutics, Stopford Building, University of Manchester, Manchester M13 9PT

Some of the effects of dipyridamole, hexobendine and lidoflazine on mechanical responses in rabbit duodenum have been investigated. In concentrations known to inhibit tissue accumulation of adenosine and its metabolites, none of these agents potentiated inhibitory responses to intramural nerve stimulation or to application of adenosine, adenosine triphosphate or phenylephrine. These results neither support nor dispute the suggestion that adenosine or a related nucleotide is the intramural inhibitory transmitter but do show that tissue accumulation in rabbit duodenum is not an important feature in the termination of the action of adenosine.

Introduction Evidence for the existence of a non-cholinergic, non-adrenergic intramural inhibitory pathway in intestinal smooth muscle has recently been reviewed (Burnstock, 1972; Furness & Costa, 1973). The suggestion that adenosine triphosphate (ATP) or a closely-related derivative is the transmitter involved was first put forward by Burnstock, Campbell, Satchell & Smythe (1970). This 'purinergic nerve' hypothesis proposed that, once released from the inhibitory neurone, the transmitter is broken down to adenosine, which is then taken up into the nerve for further transmitter synthesis. One limiting factor in accepting this hypothesis is the absence of a specific antagonist to the inhibitory action of ATP. In the absence of such an antagonist, experiments have been performed in the presence of drugs said to prevent tissue adenosine uptake. The results of such experiments in guinea-pig taenia coli are claimed to show potentiation of the effects of exogenous ATP and of intramural stimulation and to provide support for the purinergic nerve hypothesis (Satchell, Lynch, Bourke & Burnstock, 1972).

The present experiments have been performed using the intramural inhibitory pathway in rabbit duodenum (Weston, 1973) and the drugs dipyridamole, hexobendine and lidoflazine. In this tissue, these drugs seem to reduce adenosine accumulation by preventing the transformation of adenosine into its phosphate derivatives (Hulme & Weston, 1974).

Methods The effects of dipyridamole, hexobendine and lidoflazine on intramural nerve stimulation were assessed in longitudinal muscle strips bathed in Krebs solution containing atropine 10 μM, phentolamine 2.5 μM, and (±)-propranolol 4.75 μM (Weston, 1973). The effects of dipyridamole, hexobendine and lidoflazine on responses to adenosine and to ATP were measured in 2 cm lengths of whole duodenum in normal Krebs solution. The α-adrenoceptor agonist phenylephrine was used as control. Tension changes were recorded isometrically on a potentiometric recorder.

Log frequency-effect curves to intramural stimulation and log concentration-effect curves to adenosine, ATP and phenylephrine, were obtained simultaneously on control and test tissues. Test tissues were exposed to increasing concentrations of either dipyridamole, hexobendine or lidoflazine. Control tissues were not so exposed. A 4 min dose cycle was used with an agonist contact time of 30 s and with the tissue washed twice between each exposure to an agonist. Rectangular pulses of 0.5 ms duration and 30 V strength were delivered at various frequencies (1-64 Hz) for periods of 10 s every 4 minutes. Dipyridamole, hexobendine or lidoflazine was added to the Krebs solution and a 30 min equilibration period was allowed before the experiment was continued. Inhibition of mechanical activity was measured by the method of Kim, Schulman & Levine (1968).

For each experiment, the horizontal shift at the 50% level of the log frequency-effect curves and of the log concentration-effect curves was calculated by subtracting the shift in the appropriate curves from the shift observed simultaneously in the test curves. At least six experiments were performed for each concentration of dipyridamole, hexobendine and lidoflazine and the significance of the

[1] Present address: Institut für Physiologie der Universität, 355 Marburg an der Lahn, Deutschhausstrasse 2, West Germany.

mean log shifts was assessed by paired Student's *t* test. The concentrations of dipyridamole, hexobendine and lidoflazine used (10^{-8}, 10^{-7}, 10^{-6} M) were chosen from a parallel study (Hulme & Weston, 1974). In separate experiments, the agonist activity of dipyridamole, hexobendine and lidoflazine was assessed using the 4 min dose cycle described above.

Results No significant potentiation of the inhibitory action of adenosine, ATP, phenylephrine or intramural stimulation was obtained in the presence of either dipyridamole, hexobendine or lidoflazine. Significant antagonism was obtained in the case of ATP with dipyridamole (10^{-7} M), phenylephrine with hexobendine (10^{-6} M) and phenylephrine with lidoflazine (10^{-6} M). These results are summarized in Table 1.

Dipyridamole, hexobendine and lidoflazine all showed agonist activity on the rabbit duodenum. Dipyridamole (10^{-6} -5×10^{-4} M) produced an excitatory response which was rapid in onset. At 5×10^{-4} M, this excitation was followed by a marked reduction in mechanical activity which on washout gradually returned to the control level. Lidoflazine (10^{-7}-10^{-5} M) was also excitatory. However, at 10^{-4} M, all mechanical activity was abolished. Hexobendine (10^{-5}-10^{-3} M) produced an inhibition of mechanical activity.

Discussion No potentiation of inhibitory responses was seen in the present study in contrast to claims made by previous workers. It has been reported that the effects of adenosine in rabbit intestine were potentiated by dipyridamole (Stafford, 1966) and that the effects of adenosine, ATP and of intramural stimulation were potentiated in guinea-pig taenia coli by dipyridamole, and by hexobendine (Satchell *et al.*, 1972). In both cases, the published results fail to support the claims that potentiation of inhibitory responses occurred. Satchell *et al.* (1972) attempted to measure potentiation by comparing the responses to individual agonist concentrations or to a given intramural stimulation frequency before and after exposure to dipyridamole or hexobendine. Changes measured in this way are dependent on the slope of the agonist log concentration-effect curve, a small change in sensitivity in a steeply sloping curve resulting in apparently large potentiation and *vice versa*. Potentiation should be assessed by measurement of horizontal shifts in log concentration-effect curves as described in the methods section. Furthermore, in neither case were concurrent controls employed.

Dipyridamole, hexobendine and lidoflazine each produced changes in mechanical activity which may have contributed to the failure to observe potentiation of inhibitory responses. However, such changes in mechanical activity usually occurred at higher concentrations than those required to reduce adenosine accumulation and it is unlikely that this could account for the failure to observe potentiation.

The inability of dipyridamole, hexobendine and lidoflazine to potentiate the action of adenosine suggests that tissue accumulation is not an important mechanism in the termination of action of exogenous adenosine in rabbit duodenum. The results neither support nor dispute the suggestion that ATP or a related substance is the unknown inhibitory transmitter. However, Su, Bevan & Burnstock (1971) found that tritiated ATP or a breakdown product was released when either the

Table 1 Effect of dipyridamole (Dip), hexobendine (Hex) and lidoflazine (Lid) on the log frequency-effect curves to intramural stimulation and on the log concentration-effect curves to adenosine, ATP and phenylephrine

		Mean log shift with s.e. in the effect curves			
	Molar conc.	*Intramural stimulation*	*Adenosine*	*ATP*	*Phenylephrine*
Dip	10^{-8}	−0.14 ± 0.2	−0.12 ± 0.16	−0.14 ± 0.11	−0.05 ± 0.16
	10^{-7}	−0.13 ± 0.15	−0.11 ± 0.2	−0.28 ± 0.10*	−0.22 ± 0.12
	10^{-6}	−0.40 ± 0.17	−0.29 ± 0.17	−0.13 ± 0.17	−0.13 ± 0.15
Hex	10^{-8}	−0.10 ± 0.11	−0.16 ± 0.22	−0.23 ± 0.26	+0.14 ± 0.11
	10^{-7}	+0.13 ± 0.16	+0.04 ± 0.19	−0.08 ± 0.12	+0.19 ± 0.16
	10^{-6}	+0.18 ± 0.04	−0.07 ± 0.22	−0.003 ± 0.28	−0.42 ± 0.18*
Lid	10^{-8}	−0.08 ± 0.04	−0.12 ± 0.18	+0.24 ± 0.2	−0.12 ± 0.19
	10^{-7}	−0.12 ± 0.17	−0.03 ± 0.15	+0.05 ± 0.18	−0.16 ± 0.11
	10^{-6}	+0.07 ± 0.2	−0.12 ± 0.13	+0.21 ± 0.19	−0.36 ± 0.06*

* $P < 0.05$. All other values, $P > 0.05$.
Positive log shift indicates movement of curve to left, negative log shift indicates movement of curve to right.

intramural nerves or the noradrenergic nerves of guinea-pig taenia coli were stimulated. It is known that in noradrenergic nerves, noradrenaline and ATP form a complex within the storage vesicles (de Potter, 1971) and that a similar situation exists for acetylcholine and ATP in cholinergic nerves (Zimmerman & Whittaker, 1973).

These observations might suggest that ATP release on intramural nerve stimulation is not necessarily evidence that ATP itself is the inhibitory transmitter but merely that ATP is associated with the unknown transmitter substance in the storage vesicles of the inhibitory neurone.

We gratefully acknowledge gifts of the following drugs–dipyridamole (Boehringer), hexobendine (Linz) and lidoflazine (Janssen).

References

BURNSTOCK, G. (1972). Purinergic nerves. *Pharmac. Rev.*, **24**, 509-581.

BURNSTOCK, G., CAMPBELL, G., SATCHELL, D. & SMYTHE, A. (1970). Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic nerves in the gut. *Br. J. Pharmac.*, **40**, 668-688.

FURNESS, J.B. & COSTA, M. (1973). The nervous release and the action of substances which affect intestinal muscle through neither adrenoreceptors nor cholinoreceptors. *Phil. Trans. R. Soc. Lond. B.*, **265**, 123-133.

HULME, M.E. & WESTON, A.H. (1974). The accumulation of adenosine in rabbit intestinal muscle. *Br. J. Pharmac.*, **50**, 569-574.

KIM, T.S., SCHULMAN, J. & LEVINE, R.A. (1968). Relaxant effect of cyclic adenosine 3'5' monophosphate on the rabbit isolated ileum. *J. Pharmac. exp. Ther.*, **163**, 36-42.

de POTTER, W.P. (1971). Noradrenaline storage particles in splenic nerve. *Phil. Trans. Roy. Soc. Lond. B.*, **261**, 313-317.

SATCHELL, D.G., LYNCH, A., BOURKE, P.M. & BURNSTOCK, G. (1972). Potentiation of the effects of exogenously applied ATP and purinergic nerve stimulation on the guinea-pig taenia coli by dipyridamole and hexobendine. *Europ. J. Pharmac.*, **19**, 343-350.

STAFFORD, A. (1966). Potentiation of adenosine and the adenine nucleotides by dipyridamole. *Br. J. Pharmac. Chemother.*, **28**, 218-227.

SU, C., BEVAN, J.A. & BURNSTOCK, G. (1971). [^{3}H]-adenosine triphosphate: release during stimulation of enteric nerves. *Science*, **173**, 336-338.

WESTON, A.H. (1973). Nerve-mediated inhibition of mechanical activity in rabbit duodenum and the effects of desensitization to adenosine and several of its derivatives. *Br. J. Pharmac.*, **48**, 302-308.

ZIMMERMAN, H. & WHITTAKER, V.P. (1973). The biochemistry of the synaptic vesicle. *4th Pharmacological Society Symposium:* Synaptic Transmitter Substances in the Central Nervous System, School of Pharmacy, University of London.

(Received October 2, 1973)