J. Pharm. Pharmacol. 1988, 40: 521-523
Communicated January 29, 1988

Actions of amrinone and milrinone on the guinea-pig ileum

T. W. STONE, D. M. PATON*, *Department of Pharmacology and Clinical Pharmacology, School of Medicine, University of Auckland, Auckland, New Zealand*

Abstract—The pharmacological activity of the cardiotonic agents amrinone and milrinone has been examined on the isolated guinea-pig ileum. Both compounds antagonised the presynaptic inhibitory action of adenosine on electrically evoked contractions, milrinone being the more potent. Higher concentrations of amrinone or milrinone produced contractions of the ileum which were prevented by atropine. Contractile responses to adenosine 5'-monophosphate were not inhibited by atropine. It is concluded that amrinone and milrinone can antagonise some effects of purines but this does not represent their only pharmacological action.

Amrinone and milrinone are positive inotropic agents (Alousi & Johnson 1986). Part of their activity may be due to an inhibition of cyclic nucleotide phosphodiesterases (Ahn et al 1986; Endoh et al 1982; Carpenedo et al 1984), but Dorigo & Maragno (1986) have claimed that the positive inotropic activity of amrinone cannot be observed in guinea-pig isolated hearts after the inactivation of endogenous adenosine by adenosine deaminase, or the blockade of adenosine receptors by 8-phenyltheophylline. These and related observations led to the suggestion that the cardiac stimulant properties of amrinone were the result of an antagonism of endogenous adenosine.

In a subsequent report, Dorigo et al (1987) found that amrinone produced contraction of the guinea-pig ileum, followed by a relaxant effect which could be suppressed by pretreatment with theophylline. Theophylline alone, however, produced a biphasic contraction and relaxation response similar to that produced by amrinone. The present study was therefore designed to determine whether any of these responses could be attributed to antagonism of adenosine by examining responses to purines on the electrically stimulated guinea-pig ileum and by using the more selective antagonist 8-phenyltheophylline. In addition, we have compared the activity of amrinone with the more potent cardiotonic analogue, milrinone.

Methods

Female guinea-pigs were killed by exposure to an atmosphere of carbon dioxide, and the ileum removed. Approximately 2 cm lengths were cut from the middle region of the ileum and transferred to warmed, oxygenated Krebs solution of the following composition (mM): NaCl 116; KCl 5·4; NaH_2PO_4 1·2; $MgCl_2$ 1·2; $CaCl_2$ 2·5; $NaHCO_3$ 22; D-glucose 11. Each segment was placed in a 5 mL organ bath and connected to an isometric transducer. The resting tension was adjusted to 0·5 g and this was restored if necessary at intervals over the next 30 min equilibration period.

The ileum was stimulated electrically by passing pulses of 1 ms duration and 100 V amplitude between the platinum hook anchoring the tissue in the bath and a separate platinum wire. Stimuli were delivered from a Grass S48 stimulator at 0·1 Hz.

Drugs were injected into the bath in a volume of no more than 100 μL. Amrinone and milrinone were used as 25 mM solutions by dissolving in dimethylsulphoxide (DMSO). All other compounds were dissolved in distilled water. Both adenosine hemisulphate and 5'-adenosine monophosphate (AMP) were used as agonists in these experiments with qualitatively similar results. The inhibition of ileal contraction by these purines was measured as the maximum percentage decrease in twitch height. Direct effects on the tissue were highly variable and were therefore normalized, changes being expressed as the percentage of the control size.

Results

The electrically evoked twitch of the ileum was inhibited by adenosine or AMP in a concentration-dependent manner as illustrated in Fig. 1. The effects of both purines were reduced by adding amrinone or milrinone 3 min before the purine. At concentrations of 40-400 μM, amrinone displaced the purine concentration-response curves to the right in a parallel fashion (Fig. 1A). Milrinone acted similarly but was more potent, producing significant displacements of the curves at concentrations of 5 μM or above (Fig. 1B).

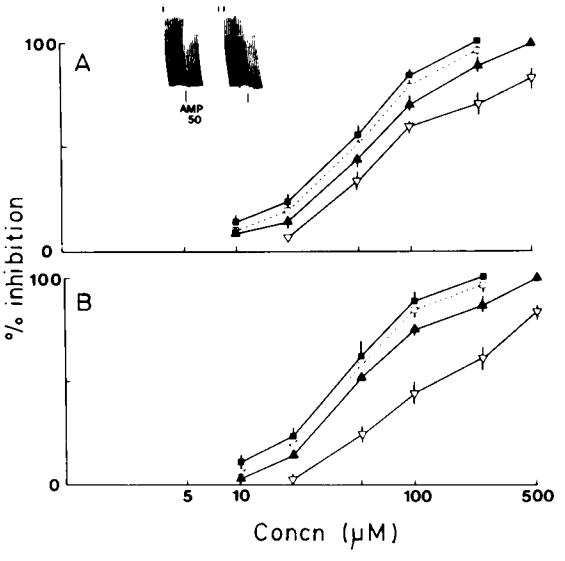

FIG. 1. Concentration-response curves for the inhibition of electrically induced twitch contractions by AMP. A. Shows a control curve (■) and rightward shifts produced by 50 (▲) and 200 μM (▽) amrinone. Inset: the inhibitory effect of AMP on the twitch responses: (i) control (ii) in the presence of amrinone 100 μM. B. Shows a control curve (■) and curves obtained in the presence of 5 (▲) and 50 μM (▽) milrinone. The interrupted lines on each graph show the curves obtained by adding 100 μL of DMSO to the organ bath. This was the maximum volume of addition when used as the solvent for amrinone and milrinone. The points show mean ± s.e.m. for at least 6 preparations, when the s.e. is larger than the symbol size.

This reduction of AMP sensitivity was not due to changes of tissue sensitivity with time. On two occasions a control preparation was tested with AMP repeatedly without treatment with amrinone or milrinone. In addition recovery of AMP sensitivity

*Present address: Department of Oral Biology, University of Alberta, Edmonton, Alberta, Canada T6G 2N8.
Correspondence and present address: T. W. Stone, Department of Physiology, St George's Hospital Medical School, University of London, London SW17 0RE, UK.

was observed after drug treatment in all the preparations used in the calculation of results.

DMSO, used to dissolve amrinone and milrinone, itself produced a small shift in the purine dose response curves though this was very variable between tissues and was not statistically significant (Fig. 1). The addition of 50 μL or more into the organ bath also produced a transient increase of basal tension and twitch height on occasions, these diminishing to control values within 4–5 min, even in the continued presence of DMSO.

The lower concentrations of amrinone and milrinone which were effective in inhibiting purine responses had no apparent effects of their own on the preparations other than the increase of tension which was attributable to DMSO. At the higher concentrations, however, a direct contractile action was noted whether the preparation was resting or electrically stimulated. This was followed by a period of diminished twitch responses to electrical stimulation (Fig. 2). These effects were noted at

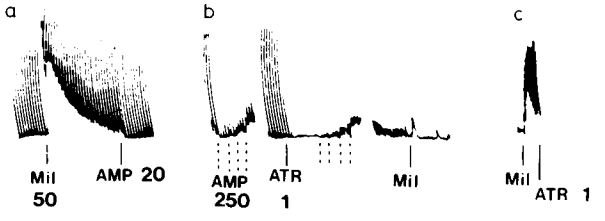

FIG. 2. A. Records of the electrically evoked twitch responses of the ileum showing the contractile effect of milrinone 50 μM and the subsequent diminution of twitch height. The depressant effect of AMP 20 μM is also shown. B. The initial twitch responses are abolished by 250 μM AMP and subsequent additions elicit weak contractions. Following washout of the nucleotide twitch height is restored, but they are then abolished by atropine, 1 μM . The contractile responses to AMP are unchanged but the response to milrinone (50 μM) has been profoundly inhibited. A and B are records from the same preparation. C. In a different preparation a contraction was induced by milrinone 100 μM and atropine (Atr) then administered into the bath at a final concentration of 1 μM. The milrinone contraction was immediately abolished.

concentrations of amrinone of 100 μM or greater, and concentrations of milrinone of 20 μM or above.

Inclusion in the bathing medium of yohimbine, 1 μM (4 preparations) or 8-phenyltheophylline 10 μM (6 preparations) had no effect on the relaxant phase of these responses, although the latter compound abolished responses to adenosine or AMP. 8-Phenyltheophylline did however reduce the size of the contractile phase. In four preparations these responses were reduced by 34% $\pm$11 (s.e.m., n=4).

The contractile responses to amrinone and milrinone seemed to be due to activation of intrinsic neurons within the ileum, since atropine 1 μM abolished these responses, together with the electrically evoked twitch (3 preparations) (Fig. 2). Contractile responses were also obtained with cumulative additions of AMP 250 μM or single additions of AMP at 5 mM or adenosine at 20 mM. These contractions were unaltered in the presence of atropine (2,3 and 5 preparations, respectively) (Fig. 2).

8-Phenyltheophylline alone (10 μM) had no effect on the basal tone of the ileum (8 preparations) although it produced a variable increase (21% $\pm$ 16 s.e.m., n = 5) in the size of electrically evoked twitches.

Discussion

We have been able to confirm the ability of amrinone to produce a contraction of the guinea-pig ileum with a subsequent reduction of basal tone, although these effects are only obtained at relatively high concentrations of the drug. The contractile components of this response appears to be due to the activation of intrinsic cholinergic neurones since they can be prevented by atropine. They are clearly distinct from nucleotide-induced contractions which do not seem to involve acetylcholine release.

However, we were unable to block the secondary relaxant components by yohimbine or by 8-phenyltheophylline at concentrations which totally suppressed purine sensitivity. We therefore conclude that this action does not involve the activation of purine receptors or presynaptic α_2-adrenoceptors.

Furthermore, 8-phenyltheophylline alone had no effect on basal tension in the ileum, from which we suggest that amrinone was not producing these effects by antagonizing endogenous adenosine. An observation we cannot explain, however, is the ability of 8-phenyltheophylline to reduce contractile effects of the test drugs. One possibility is that this action results from stimulation of adenosine receptors, since adenosine can cause contraction of the ileum in high concentrations (Dorigo et al 1987). However, the amrinone contraction can be blocked by atropine, whereas adenosine contractions cannot, as reported above.

At much lower concentrations both amrinone and milrinone were able to reduce the inhibitory effect of purines on nerve terminals in the ileum, and this antagonistic action was competitive, judged by the parallel shift of the concentration response curves. The mechanism of the interaction is unclear from the present data. There may be direct antagonism at the purine receptors, or there may be a functional antagonism at a post-receptor level. Adenosine probably produces its inhibition of transmitter release by suppressing depolarization-induced calcium influx into nerve terminals (see Stone 1981) and it may therefore be relevant that amrinone and milrinone can promote calcium influx, at least into preparations of cardiac tissue (Sutko et al 1986).

It is therefore concluded that the effects of amrinone and milrinone on the ileum are complex but that at low micromolar concentrations both agents can reduce adenosine responses. At high levels new effects are produced which are largely unrelated to purine receptors. It is not clear which, if any, of these various actions may be relevant to the cardiotonic properties of the drugs, but it is interesting to note that milrinone was approximately ten-fold more potent than amrinone both when inhibiting presynaptic actions of purines and when directly affecting the ileum. A similar ratio pertains to their cardiostimulant activity (Alousi & Johnson 1986).

This work was supported by the New Zealand M.R.C. and the award of a Wellcome Trust/New Zealand M.R.C. travel grant to T.W.S. The provision of amrinone and milrinone by Sterling-Winthrop is gratefully acknowledged.

References

Ahn, H. S., Eardly, D., Watkins, R., Prioli, N. (1986) Effects of several cardiotonic drugs on cardiac cyclic AMP metabolism. Biochem. Pharmacol. 35: 1113–1121

Alousi, A. A., Johnson, D. C. (1986) Pharmacology of the bipyridines: amrinone and milrinone. Circulation 73, Suppl III:III 10–24

Carpenedo, F., Floreani, M., Cargnelli, G. (1984) Competitive inhibition of phosphodiesterase activity by amrinone: its implication in the cardiac effect of the drug. Pharmacol. Res. Comm. 16: 969–977

Dorigo, P., Maragno, I. (1986) Interaction of amrinone with endogenous adenosine in guinea-pig atria. Br. J. Pharmacol. 87: 623–629

Dorigo, P., Gaion, R. M., Giacometti, A., Ceroni, G., Maragno, I.

(1987) Possible role of adenosine in the relaxant effect of amrinone on guinea-pig ileum. J. Autonom. Pharmacol. 7: 53–60

Endoh, M., Yamashita, S., Taira, N. (1982) Positive inotropic effect of amrinone in relation to cyclic nucleotide metabolism in the canine ventricular muscle. J. Pharmacol. Exp. Ther. 221: 775–783

Stone, T. W. (1981) Physiological roles for adenosine and ATP in the nervous system. Neuroscience 6: 523–555

Sutko, J. L., Kenyon, J. L., Reeves, J. P. (1986) Effects of amrinone and milrinone on calcium influx into the myocardium. Circulation 73, Suppl. III: III 52 58

J. Pharm. Pharmacol. 1988, 40: 523–524
Communicated January 11, 1988

Dopamine-mediated behaviour following chronic treatment with B-HT 920

N. A. DARMANI, R. D. E. SEWELL, P. J. NICHOLLS *The Welsh School of Pharmacy, UWIST, P.O. Box 13, Cardiff CF1 3XF, UK*

Abstract—Following subchronic (5-day) dosing with B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-4*H*-thiazolo(4,5-*d*)azepine (1 mg kg^{-1} day^{-1} i.p.) in rats there was a significant increase in both apomorphine-induced motor activity and stereotypy. On continued B-HT 920 treatment, however, the enhancement of apomorphine motor activity faded into insignificance but the increase in stereotypy persisted beyond 15 days. The results are discussed in terms of dopamine autoreceptor tolerance, postsynaptic D_2 supersensitivity and possible differential effects in different brain loci on the above two receptor sub-classes.

B-HT 920 is an azepine derivative shown to have agonist effects on central and peripheral α_2-adrenoceptors causing hypotension and bradycardia (Kobinger & Pichler 1980; Pichler & Kobinger 1981). B-HT 920 also produces a decrease in motor activity in mice and this is thought to be a consequence of α_2-adrenoceptor activation. However, subsequent studies (Anden et al 1982, 1983) have revealed that, in addition, B-HT 920 possesses central dopamine (DA) agonist activity with marked selectivity for autoreceptors at low doses. B-HT 920 at such dose levels not only inhibits locomotion in mice but also decreases the firing rate in dopaminergic neurons (Eriksson et al 1985). Furthermore, it produces a dose-dependent retardation of α-methyl-*p*-tyrosine-induced reduction of DA content in rat brain and inhibition of γ-butyrolactone-stimulated DA synthesis in rat corpus striatum. These effects are antagonized by spiperone and haloperidol (Brown et al 1984; Mierau & Schingnitz 1987). There is no effect, however, upon DA-sensitive adenylate cyclase, suggesting that B-HT 920 does not act at D_1-receptors. Indeed its pharmacological effects are characteristic of a dopamine D_2-autoreceptor agonist (Brown & Mitchell 1985). However, if the presynaptic dopaminergic terminals have degenerated, additional postsynaptic effects of B-HT 920 emerge and become predominant (Mierau & Schingnitz 1987). Grabowska-Anden & Anden (1987) have also demonstrated a direct action of B-HT 920 on postsynaptic D_2 receptors (head jerks) when D_1 receptors are antagonized by SCH 23390. Following low chronic dosing, the autoagonistic effects of B-HT 920 may lead to postsynaptic D_2-receptor supersensitivity arising from prolonged inhibition of dopamine release. This study was therefore undertaken to investigate the chronic effects of B-HT 920 on apomorphine-induced motor activity and stereotypy as behavioural measures of dopamine receptor sensitivity.

Materials and methods

Male Wistar rats (150 g) were housed on a 12 h light- 12 h dark cycle and allowed free access to food and water. The animals were dosed daily with either B-HT 920 (1 mg kg^{-1} i.p. n = 6) or 0·9% NaCl (saline) (n = 6) for periods of 5 and 15 days. The experiments were carried out 36 h after the last dose. One hour after habituation to test environment the animals received apomorphine (0·5 mg kg^{-1} i.p.). Then, cumulative motor activity was measured via electronic counters every 10 min for a period of 1 h using photocell cages measuring 48 × 26 × 26cm. Paired cages were fitted with three light beams (one along the long axis and two equally spaced along the short axis of the oblong floor space. Single animals (test and control) were studied concurrently and simultaneously stereotyped behaviour was observed and assessed for 2 min in each 10 min period using the scoring system employed by Creese & Iversen (1973). Statistical analysis of difference between means was determined using Student's *t*-test.

The drugs used were: apomorphine hydrochloride HCl (Sigma), B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-4*H*-thiazolo(5,4-*d*)azepine 2HCl (Boehringer Ingelheim).

Results

In subchronic studies (5 days treatment) B-HT 920 caused a significant increase ($P < 0.02$) in motor activity between the 10-20 min time period, the motor activity count being 174·8% of saline-pretreated controls, and this effect persisted throughout the experiment. In chronic studies (15 days treatment) no such locomotor change ($P > 0.05$) was observed between B-HT 920 and controls at any time period after apomorphine injection (Fig. 1).

In subchronically B-HT 920-treated rats, a significant increase (160% $P < 0.04$) in stereotyped behaviour was seen between 10 and 20 min. However, in 15 day chronically treated animals the increase in stereotyped behaviour persisted up to 50 min after apomorphine injection compared with saline-pretreated controls (50–78·5%, $P < 0.03$) (Fig. 2).

Discussion

Creese & Iversen (1973) provided behavioural evidence for post-synaptic supersensitivity following biochemical disruption of dopamine neuronal terminals using 6-hydroxydopamine. Since B-HT 920, at low doses through its autoreceptor action, inhibits the release of dopamine from presynaptic terminals, it should also cause a similar post-synaptic supersensitivity on repeated dosage to that described above. This is evidenced by increased apomorphine-induced locomotor activity and stereotyped behaviour after five daily treatments with the dopamine autoreceptor agonist in our study. However, if the drug dosing regimen is extended to 15 days, the increase in sensitivity to apomorphine locomotor activity fades into insignificance. This loss of dopamine supersensitivity between 5 and 15 day B-HT 920 treatment

Correspondence to: N. A. Darmani, The Welsh School of Pharmacy, UWIST, P.O. Box 13, Cardiff CF1 3XF, UK.