tion and therefore might have an impact on final cell destruction.

To sum up, during reperfusion of the isolated rat brain ADP, glucose-6-phosphate, and lactate levels were lowered by nimodipine. The calcium antagonist caused no change in preischemic flow rate but increased postischemic perfusion rate considerably. Lactate levels were unrelated to the corresponding flow values. The results suggest nimodipine to improve postischemic mitochondrial function by a direct action on cerebral parenchyma.

Acknowledgements—The authors are indebted to Mrs M. Lorenz for her skilful technical assistance.

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Biochemical Pharmacology, Vol. 38, No. 5, pp. 855–858, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

Amrinone potentiates catecholamine-induced lipolysis in fat cells

(*Received* 15 *September* 1987; *accepted* 31 *August* 1988)

Amrinone, a bipyridine derivative, exerts a positive inotropic action *in vitro* [1–3] and *in vivo* [4]. In addition, the drug produces a marked relaxation of vascular [5, 6] and intestinal [5, 7–9] smooth muscle.

In patients with cardiac failure the haemodynamic effects of amrinone are accompanied by an increase in plasma fatty acids and glycerol concentration [10].

The present study was undertaken in order to test the possibility that amrinone may exert a direct stimulatory effect on hormone-sensitive lipolysis in fat cells. This hypothesis is based on the consideration that amrinone inhibits phosphodiesterase activity [3, 11, 12] and hinders the response to endogenous adenosine [2, 9] in various tissues. In fat cells, cyclic AMP formation is an intermediate step in the activation of triglyceride lipase by hormones and neurotransmitters interacting with stimulatory receptors [13]. This activation can be also induced by agents, such as methylxanthines, that act as antagonists at inhibitory membrane receptors coupled to adenylate cyclase and/or increase cyclic AMP levels by inhibiting phosphodiesterase [13–15].

Because of these similarities between the actions of amrinone and of theophylline, a typical lipolytic methylxanthine, the influence of the two drugs on lipolysis was compared and their possible interaction at this level was examined.

Finally, since activation of Ca^{2+} flux in vascular [5], intestinal [8, 16] and cardiac muscle [3, 17] is involved in the final effects of amrinone, and many results indicate an outstanding role of Ca^{2+} in the regulation of lipolysis [18, 20], the influence of external Ca^{2+} concentration on the effects of amrinone was also tested.

Methods

Epididymal fat pads were removed from male Wistar rats (180–240 g b.w.) under light ether anaesthesia and

immediately washed in Krebs–Ringer bicarbonate (K–Rb) buffer pH 7.4, containing 3% bovine serum albumin fraction V (Sigma Chemical Co., St. Louis, MO). Where indicated, the $CaCl_2$ concentration of the K–Rb buffer was reduced from 2.7 mM to 0.27 mM. Fat cells were isolated as previously described [19] and the final cell suspension was diluted in order to obtain a concentration of 25,000–50,000 cells/ml. The number of cells was estimated by the dilution method and counting under microscope as described by Rodbell and Krishna [21]. Aliquots of 2 ml of the cell suspension were preincubated for 15 min in a metabolic shaker at 37° in the absence or presence of amrinone (Schiapparelli, Italy), adenosine (Sigma Chemical Co., St Louis, MO) or adenosine deaminase (Sigma Chemical Co., St Louis, MO). (−)Noradrenaline bitartrate (Sigma Chemical Co., St Louis, MO) and/or theophylline (Sigma Chemical Co., St Louis, MO) were then added where indicated and the samples further incubated for 30 min. The incubation was terminated by the addition of 0.2 ml of 50% trichloroacetic acid and the previously described procedure [19] was used for the quantitation of glycerol. Amrinone was first dissolved in 0.5 M HCl and subsequent dilutions were performed in saline (NaCl 0.9%) and added to the incubation medium in volumes of 0.01 ml. The pH of the K–Rb buffer was lowered at final amrinone concentrations of 10^{-4} M and higher. Thus the maximum concentration used was 8×10^{-5} M.

The statistical significance of the changes induced by drugs was calculated by the Student's *t*-test.

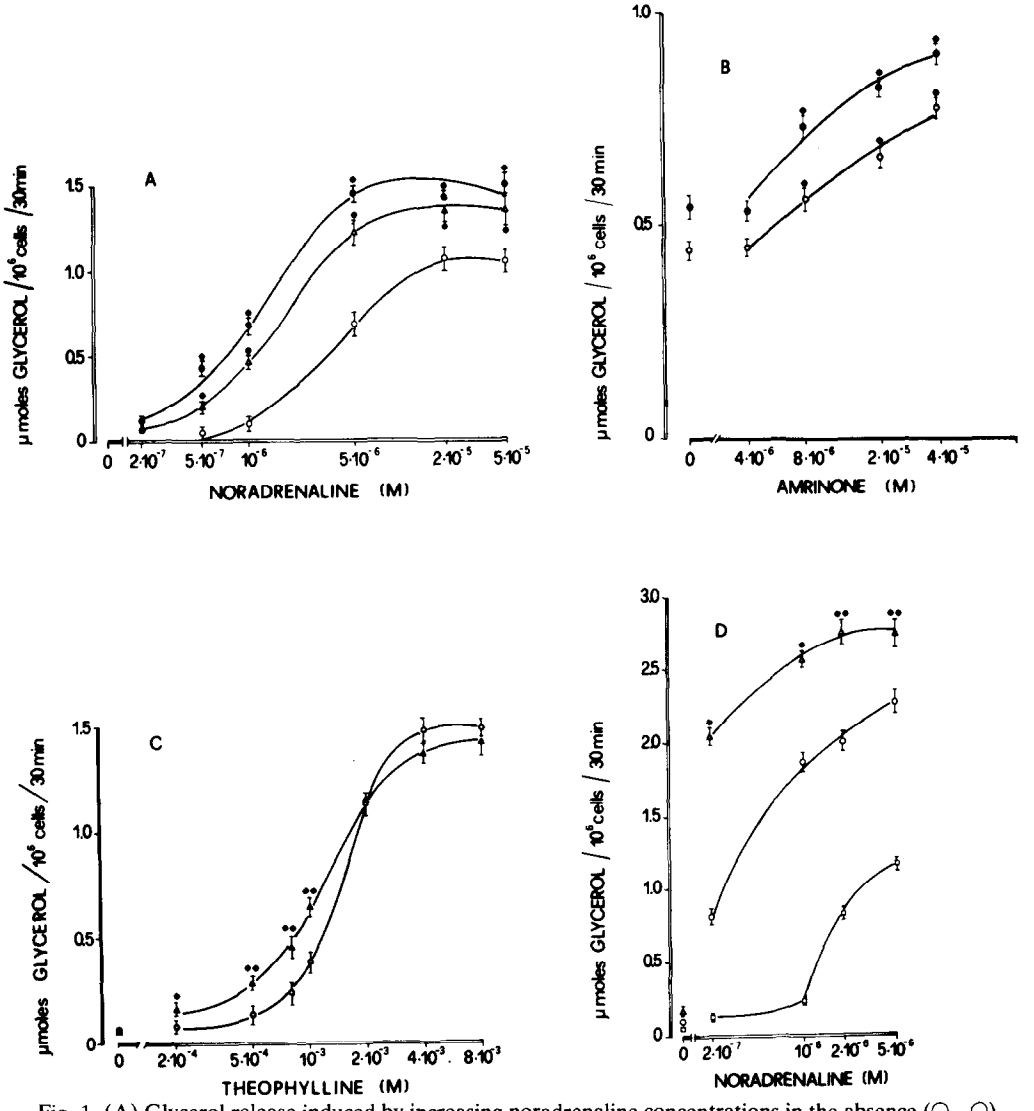

Fig. 1. (A) Glycerol release induced by increasing noradrenaline concentrations in the absence (○—○) and presence of amrinone 4×10^{-6} M (△—△) and 4×10^{-5} M (●—●). Each value is the mean (±SEM) of the results obtained from 6–9 incubations in 4 experiments. *$P < 0.001$. (B) Effect of amrinone on glycerol release induced by theophylline 8×10^{-4} M (○—○) and 10^{-3} M (●—●). Each value represents the mean (±SEM) of the data obtained from 6–10 incubations in four experiments. *$P < 0.001$. (C) Glycerol release induced by increasing theophylline concentrations in the absence (○—○) and presence (▲—▲) of 4×10^{-5} M amrinone. Each value is the mean (±SEM) of the results obtained from 8–10 incubations in three experiments. *$P < 0.02$; **$P < 0.001$. (D) Glycerol release induced by increasing noradrenaline concentrations in the absence (□—□) and in the presence of theophylline 5×10^{-4} M (○—○) or theophylline 5×10^{-4} M plus amrinone 4×10^{-5} M (△—△). Each value represents the mean (±SEM) of the data obtained from 6–8 incubations in three experiments. *$P < 0.001$; **$P < 0.005$.

Table 1. Influence of adenosine on noradrenaline-induced glycerol release in the absence and presence of amrinone

Drugs (M)	Glycerol (μmoles/10^6 cells/30 min)			
	Control	P	Amrinone 4×10^{-5} M	P
Noradrenaline 2×10^{-6}	0.57 ± 0.05		1.14 ± 0.09	
+ Adenosine 10^{-7}	0.47 ± 0.03	<0.02	1.08 ± 0.17	ns
+ Adenosine 10^{-6}	0.38 ± 0.02	<0.001	1.00 ± 0.09	ns
+ Adenosine 10^{-5}	0.36 ± 0.02	<0.001	1.06 ± 0.10	ns
+ Adenosine 10^{-4}	0.32 ± 0.02	<0.001	0.91 ± 0.09	<0.01
+ Adenosine 5×10^{-4}	0.30 ± 0.02	<0.001	0.79 ± 0.05	<0.005

Adenosine and amrinone were added at the beginning of the 15 min preincubation. The incubation was carried out for 30 more min in the presence of noradrenaline. Each value is the mean (±SEM) of the data obtained from 9–10 incubations in four experiments. The statistical significance of the changes caused by adenosine on noradrenaline-induced lipolysis in the absence and in the presence of amrinone was calculated by the Student's *t*-test. ns = not statistically significant.

Results

Amrinone, at concentrations from 2×10^{-6} to 8×10^{-5} M, did not alter the basal rate of glycerol release from fat cells incubated either in normal (2.7 mM) or in low $CaCl_2$ (0.27 mM) medium (not shown).

As shown in Fig. 1A, amrinone (4×10^{-6} and 4×10^{-5} M) potentiated the lipolytic response to noradrenaline at all the catecholamine concentrations tested (2×10^{-7}–5×10^{-5} M). Reduction of the $CaCl_2$ concentration in incubation medium from 2.7 mM to 0.27 mM, markedly decreased the absolute rate of noradrenaline (2×10^{-7}–2×10^{-5} M)-activated lipolysis (e.g. at 2×10^{-6} M noradrenaline glycerol release was 0.76 ± 0.02 and 0.38 ± 0.04 μmoles/10^6 cells/30 min, respectively). Also the per cent response to the catecholamine was reduced (+633% and +387%, respectively), whereas the potentiating action of 4×10^{-5} M amrinone, expressed as per cent increase over noradrenaline effect, changed only slightly (+273% and +312%, respectively).

As illustrated in Fig. 1B, amrinone (8×10^{-6}–4×10^{-5} M) exerted a concentration-dependent potentiating effect on glycerol release stimulated by theophylline. This potentiation was statistically significant at submaximally effective concentrations of the xanthine (2×10^{-4}–10^{-3} M), while the response to the highest ones was unaffected by amrinone (Fig. 1C). External Ca^{2+} lowering (from 2.7 to 0.27 mM $CaCl_2$) reduced the absolute lipolytic activity of theophylline both in the absence and in the presence of amrinone. However, the per cent increases of lipolysis induced by theophylline over the basal rate (147% vs 151% increase by 5×10^{-4} M theophylline), as well as the potentiating effect of 4×10^{-5} M amrinone, expressed as per cent stimulation of theophylline effect (+93% vs +100%), were not consistently altered (not shown).

Theophylline, at a concentration that had a negligible effect on spontaneous lipolysis (5×10^{-4} M), increased the response of fat cells to all the concentrations of noradrenaline tested (2×10^{-7}–5×10^{-6} M) (Fig. 1D). Under these conditions, i.e. in the presence of both noradrenaline and theophylline, the addition of 4×10^{-5} M amrinone caused a further stimulation of glycerol release (Fig. 1D).

Adenosine deaminase (0.5 U/ml) exerted the well known stimulatory effect both on basal and on catecholamine-induced glycerol release. In fat cells treated with adenosine deaminase, amrinone (4×10^{-5} M) did not cause any significant change in the effect of noradrenaline (5×10^{-7}–5×10^{-6} M). Similarly, preincubation of fat cells with this enzyme increased the lipolytic response to theophylline and abolished the influence of amrinone on methylxanthine-stimulated glycerol release (not shown).

Exogenous adenosine (10^{-7}–5×10^{-4} M) did not influence spontaneous lipolysis or glycerol release from fat cells incubated with amrinone (not shown), but inhibited noradrenaline (2×10^{-6} M)-induced lipolysis (Table 1). In the presence of amrinone (4×10^{-5} M), the potentiation of noradrenaline effect caused by the drug was reduced only by the highest adenosine concentrations tested (Table 1).

Discussion

The results of the present study show that low, therapeutically relevant concentrations of amrinone exert a stimulatory effect on catecholamine-induced lipolysis in rat fat cells, in accordance with its ability to increase plasma fatty acid and glycerol levels *in vivo* [10].

Under physiological conditions lipid mobilization from adipose tissue is tightly controlled by the adrenergic system and some local modulators, such as adenosine and prostaglandins, play a primary role in the final lipolytic response to adrenergic stimulation [13, 22]. The ability of amrinone to potentiate noradrenaline effect indicates that the drug may act at some site of the lipolytic mechanism that couples receptor-mediated signals to the final metabolic response. Alternatively, amrinone might remove an endogenous inhibitor of catecholamine-induced lipolysis [22] or act at some step of the lipolytic cascade distal to adenylate cyclase activation [13].

In the attempt to obtain some evidence that could allow discrimination among these possibilities, the interaction between amrinone and theophylline was tested both in the absence and in the presence of noradrenaline.

Theophylline increases the basal rate of lipolysis by blocking adenosine receptors [14, 15]. Amrinone potentiated this effect, without altering maximum lipolytic responses to this methylxanthine. Moreover amrinone, like theophylline, potentiated the lipolytic response to noradrenaline at any catecholamine concentration tested and the two drugs exerted additive effects at this level. These results stress that some similarities may exist between the mechanisms underlying the effects of amrinone and of theophylline.

Amrinone was ineffective in the presence of adenosine deaminase, showing that the ability of amrinone to potentiate catecholamine- and theophylline-induced lipolysis is strictly dependent on the presence of endogenous adenosine. Moreover, the concentrations of exogenous adenosine required for inhibiting noradrenaline-induced lipolysis were much higher in the presence than in the absence of 4×10^{-5} M amrinone, further suggesting a competitive interaction between adenosine and amrinone. This interpretation is in keeping with the ability of amrinone to bind to adenosine receptors in the heart [3] and in fat cells

[23] and to affect the activity of guinea pig atria [2], ileum [9, 24] and aorta [25] by interfering with endogenous adenosine.

The inhibition of lipolysis by adenosine is not influenced by extracellular Ca^{2+} concentration [26, 27]. Thus, if amrinone acts by preventing the antilipolytic effect of endogenous adenosine, the final response to amrinone will also be very likely independent from external Ca^{2+}. Even though the absolute rate of lipolysis induced by amrinone associated with either noradrenaline or theophylline was decreased by reducing external Ca^{2+} concentration, the ability of amrinone to induce a given per cent increase over noradrenaline or theophylline effect was not altered by Ca^{2+}. Similarly, the relative increase of basal lipolysis induced by theophylline was not consistently modified by reducing extracellular Ca^{2+} concentration, suggesting that Ca^{2+} is required for the optimal amplification of lipolytic signals, but it does not affect the specific site(s) of amrinone and of theophylline action.

Independently from the specific mechanism of action, the ability of amrinone to amplify the lipolytic response to noradrenaline is likely to contribute to the increase of plasma fatty acids and glycerol following amrinone administration *in vivo* [10].

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