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# Protective Effects of Dilazep and its Derivative K-7259 on the Haemolysis Induced by Amphiphiles in Rat Erythrocytes

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## Abstract

The effects of dilazep and K-7259, a dilazep derivative, on the haemolysis (as evidenced by release of haemoglobin) induced by palmitoyl-L-carnitine (PAL-CAR) or palmitoyl 1-α-lysophosphatidylcholine (PAL-LPC) have been determined in rat erythrocytes.

At concentrations above the critical micelle concentration both PAL-CAR and PAL-LPC induced haemolysis; the concentrations of PAL-CAR and PAL-LPC producing 50% haemolysis were approximately 13 and 14 μM, respectively. The 50% haemolysis induced by PAL-CAR or PAL-LPC was attenuated by dilazep (1, 10 or 100 μM) but not at the highest concentration used (1 mM). K-7259 attenuated the 50% haemolysis induced by PAL-CAR or PAL-LPC at concentrations ranging from 1 μM to 1 mM. Similarly, dilazep (1 to 100 μM) and K-7259 (1 μM to 1 mM) significantly or insignificantly attenuated the 25% and 75% haemolysis induced by PAL-CAR or PAL-LPC. Neither dilazep nor K-7259 affected micelle formation by PAL-CAR or PAL-LPC, nor, at concentrations of 1 and 10 μM, did they attenuate the haemolysis induced by osmotic imbalance (hypotonic haemolysis).

These results suggest that both dilazep and K-7259 protect the erythrocyte membrane from the damage induced by PAL-CAR or PAL-LPC. The protective effects of dilazep and K-7259 are mediated by some mechanism other than prevention of micelle formation or protection of the erythrocyte membrane against osmotic imbalance.

Dilazep is known to attenuate the mechanical dysfunction and metabolic changes of the heart induced by ischaemia and reperfusion (Sugiyama et al 1985; Hoque et al 1995a). The anti-ischaemic action of dilazep has been considered to result in potentiation of the cardiovascular effects of adenosine (Mustafa 1979; Van Belle 1993). Recently K-7259 (*N,N'*-bis[4-(3,4,5-trimethoxyphenyl)butyl]homopiperazine dihydrochloride), a dilazep derivative, was also shown to attenuate myocardial derangements induced by ischaemia-reperfusion (Hoque et al 1995b), although its potentiating action on the adenosine-mediated effects was very weak—approximately one thousandth that of dilazep (Hara et al 1995). According to Hoque et al (1995a, b), the cardioprotective effect of K-7259 against ischaemia-reperfusion damage is more potent than that of dilazep in the isolated perfused heart. Therefore, mechanisms other than potentiation of adenosine-mediated cardiovascular effects might contribute to protection of the myocardium from ischaemia-reperfusion damage by dilazep or K-7259, or both (Hara & Abiko 1996a; Hara et al 1996).

Recently, we found that dilazep and K-7259 attenuate mechanical and metabolic derangements induced by amphiphilic metabolites such as palmitoyl-L-carnitine (PAL-CAR) and palmitoyl 1-α-lysophosphatidylcholine (PAL-LPC) in the rat heart (Hoque et al 1995c; Hara et al 1996). The action of dilazep and K-7259 in attenuating these derangements might contribute to their protection of the myocardium from ischaemia-reperfusion damage, because PAL-CAR and PAL-LPC are factors causing ischaemia-reperfusion damage. According to pathophysiological studies, PAL-CAR and PAL-LPC cause severe damage to the sarcolemmal membrane, because of their amphiphilic properties, and hence induce myocardial derangements (Corr et al 1987; Datorre et al 1991). Nevertheless, it is unclear whether dilazep and K-7259 protect the cell membrane itself from the damage induced by PAL-CAR and PAL-LPC. The haemolytic action of PAL-CAR and PAL-LPC has been regarded as a typical example of damage to biological membranes (Cho & Proulx 1971; Utsumi et al 1978; Bierbaum et al 1979; Weltzien 1979; Bergmann et al 1981).

It has been demonstrated that some anti-ischaemic drugs have so-called 'membrane-stabilizing action', which might contribute to their protection of the myocardium against ischaemic damage (Hara & Abiko 1996b). The membrane-stabilizing action of drugs has been evaluated extensively in the erythrocyte. For example, propranolol, nicardipine, diltiazem and lidocaine protect the erythrocyte membrane against hypotonic haemolysis (Sasaki et al 1984; Rogers et al 1986; Abe et al 1991). Furthermore, anti-ischaemic drugs are shown to reduce haemolysis induced by amphiphilic metabolites. Neufeld et al (1985) have shown that lidocaine attenuates the haemolysis induced by PAL-LPC in the canine erythrocyte. Anderson et al (1996) have reported that propranolol attenuates PAL-LPC-induced haemolysis in the sheep erythrocyte. PAL-CAR, an amphiphilic metabolite that is structurally similar to PAL-LPC, also causes irreversible damage in the ischaemic heart (Corr et al 1987; Datorre et al 1991) and severe haemolysis in the erythrocyte (Cho & Proulx 1971; Piper et al 1984). Therefore, the erythrocyte haemolysis induced by PAL-CAR or PAL-LPC would be a useful model for evaluation of the membrane-stabilizing action of anti-ischaemic drugs.

This study was performed to investigate the effects of dilazep and K-7259 on the haemolysis induced by PAL-CAR or PAL-LPC in the erythrocyte. We used erythrocytes from rats, because the cardioprotective action of dilazep and K-7259

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on PAL-CAR or PAL-LPC was demonstrated in the rat (Hoque et al 1995c; Hara et al 1996).

## Materials and Methods

*Materials*

Dilazep and K-7259 were kindly supplied by Kowa Pharmaceutical Company (Tokyo, Japan). PAL-CAR and PAL-LPC were purchased from Sigma (St Louis, MO), and 8-anilino-1-naphthalenesulphonic acid was obtained from Tokyo Kasei Kogyo (Tokyo, Japan).

*Blood sampling*

The male Sprague-Dawley rats (Sankyo Labo Service Corporation, Sapporo, Japan), 310–340 g, used in the study had free access to food and water. They were injected with heparin (1000 units $kg^{-1}$, i.p.) 30 min before induction of anaesthesia. Whole blood was collected under anaesthesia with sodium pentobarbitone (50 mg $kg^{-1}$, i.p.). The blood sample was washed three times with isotonic buffer (10 mM phosphate buffer containing 154 mM NaCl, adjusted to pH 7·4) to remove buffy coat and plasma, and 40% (v/v) erythrocyte suspension was finally obtained.

*Experimental procedures*

In the first series of experiments, the effects of dilazep and K-7259 on PAL-CAR- or PAL-LPC-induced haemolysis were examined. The erythrocyte suspension (200 μL) was mixed with the isotonic buffer (1 mL) containing dilazep or K-7259 (1, 10, 100 or 1000 μM) and incubated for 30 min at 37°C. The mixture (200 μL) of erythrocyte and dilazep was added to isotonic buffer (5 mL) containing dilazep and PAL-CAR or dilazep and PAL-LPC to produce one set of samples. Similarly, the mixture (200 μL) of erythrocyte and K-7259 was added to isotonic buffer (5 mL) containing K-7259 and PAL-CAR or K-7259 and PAL-LPC to make another set of samples. The concentration of dilazep or K-7259 in the samples was 1, 10, 100 or 1000 μM; that of PAL-CAR was 9, 13 or 18 μM and that of PAL-LPC was 10, 14 or 18 μM. Each sample was incubated for 30 min at 37°C and centrifuged at 2500 rev $min^{-1}$ for 5 min. The preliminary experiment was performed to determine the extent of haemolysis in response to different concentrations of PAL-CAR or PAL-LPC. The erythrocyte suspension (1 mL) was diluted with the isotonic buffer solution (5 mL) and incubated for 30 min at 37°C. The diluted suspension (200 μL) was mixed with the isotonic buffer (5 mL) containing different concentrations of PAL-CAR or PAL-LPC. Each of the resulting mixtures (samples) was incubated for 30 min at 37°C and centrifuged at 2500 rev $min^{-1}$ for 5 min.

The second series of the experiments was performed to determine the effects of dilazep and K-7259 on hypotonic haemolysis. The erythrocyte suspension (200 μL) was mixed with the isotonic buffer (1 mL) containing dilazep or K-7259, and incubated for 30 min at 37°C. The mixture (200 μL) of erythrocyte and dilazep was added to the hypotonic buffer (10 mM phosphate buffer containing 54, 59 or 63 mM NaCl, adjusted to pH 7·4; 5 mL) containing dilazep. Similarly, the mixture (200 μL) of erythrocyte and K-7259 was added to the hypotonic buffer (5 mL) containing K-7259. The concentration of dilazep or K-7259 in the isotonic and hypotonic buffers was 1, 10, 100 or 1000 μM. Each of the resulting samples was incubated for 30 min at 37°C and centrifuged at 2500 rev $min^{-1}$ for 5 min. The preliminary experiment was performed to determine the extent of haemolysis in response to different concentrations of NaCl. The erythrocyte suspension (1 mL) was diluted with the isotonic buffer solution (5 mL) and incubated for 30 min at 37°C. The diluted suspension (200 μL) was mixed with buffer (5 mL) containing different concentrations of NaCl. Each of the resulting samples was incubated for 30 min at 37°C and centrifuged at 2500 rev $min^{-1}$ for 5 min.

After centrifugation the extent of haemolysis in each of the experiments was determined from the absorbance of haemoglobin released into the supernatant; this was measured by spectrophotometry (Jasco Co, V-520, Tokyo, Japan) at 540 nm. The maximum haemolysis obtained in deionized water was taken as 100%, and the minimum (spontaneous) haemolysis obtained in the isotonic buffer was taken as 0%. Neither dilazep nor K-7259 itself interfered with measurement of the absorbance.

*Determination of micelle formation*

We examined whether dilazep or K-7259 affected micelle formation by PAL-CAR or PAL-LPC. The extent of micelle formation was measured by the fluorescence method using 8-anilino-1-naphthalenesulphonic acid (Ortner et al 1979). Isotonic buffer (10 mM phosphate buffer containing 154 mM NaCl, adjusted to pH 7·4) containing 8-anilino-1-naphthalenesulphonic acid (10 μM) and different concentrations of PAL-CAR or PAL-LPC was incubated for 30 min at 37°C. Similar procedures were performed in the presence of dilazep (100 μM) or K-7259 (100 μM). The intensity of 8-anilino-1-naphthalenesulphonic acid fluorescence was measured using excitation and emission wavelengths of 375 and 480 nm, respectively, with a Hitachi (Tokyo, Japan) model 204-R fluorescence spectrophotometer. The intensity of fluorescence of 8-anilino-1-naphthalenesulphonic acid obtained in isotonic buffer containing PAL-CAR (20 μM) or PAL-LPC (20 μM) was taken as 100%, and that in PAL-CAR-free or PAL-LPC-free isotonic buffer was taken as 0%. Neither dilazep nor K-7259 itself interfered with the measurement of fluorescence.

*Statistical analysis*

All values are expressed as mean ± the standard error of the mean (s.e.m.). Statistical analysis of results was performed by analysis of variance followed by Dunnett's test for comparison of the control group with each of the drug-treated groups. A value of $P<0.05$ was considered to be indicative of statistical significance.

## Results

*Effect of dilazep and K-7259 on haemolysis induced by PAL-CAR or PAL-LPC*

Fig. 1 shows the extent of haemolysis in response to various concentrations of PAL-CAR ($n=4$ at each concentration) and PAL-LPC ($n=4$ at each concentration). Both PAL-CAR and PAL-LPC caused haemolysis in a concentration-dependent manner. The concentrations of PAL-CAR inducing 25% (mild), 50% (moderate) and 75% (severe) haemolysis were approximately 9, 13 and 18 μM, respectively; for PAL-LPC the

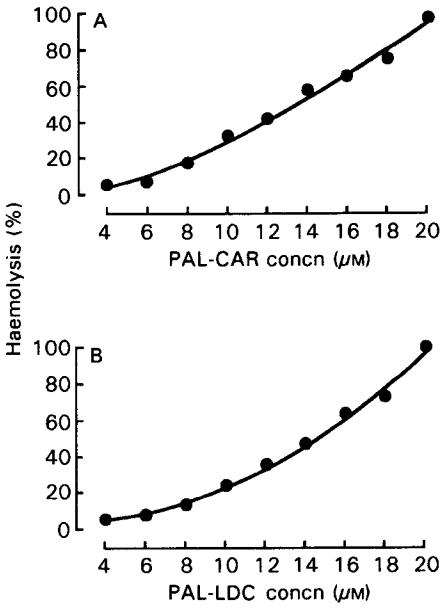


FIG. 1. The extent of haemolysis in response to different concentrations of PAL-CAR (A) or PAL-LPC (B). The mixture of erythrocytes and PAL-CAR or erythrocytes and PAL-LPC was incubated in isotonic buffer (10 mM phosphate buffer containing 154 mM NaCl; pH 7·4) for 30 min at 37°C. The maximum haemolysis obtained in deionized water was taken as 100% and the minimum (spontaneous) haemolysis obtained in the isotonic buffer was taken as 0%. Each value is the mean ± s.e.m. of results from four samples for each concentration of PAL-CAR or PAL-LPC.

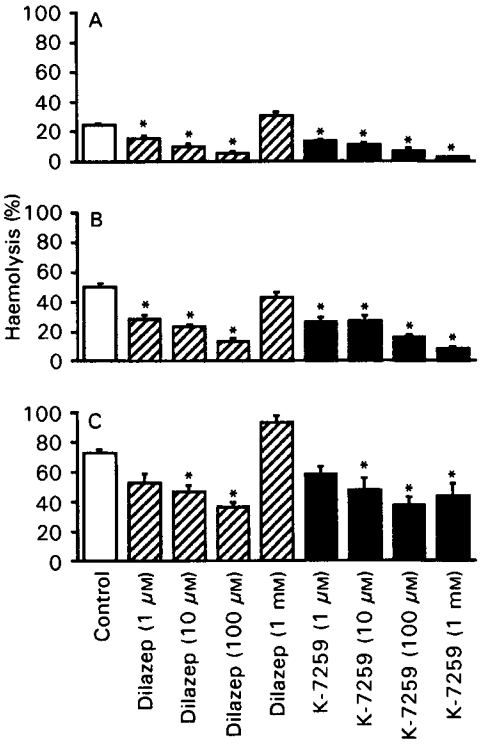


FIG. 2. Effects of dilazep and K-7259 on the mild (A), moderate (B) and severe (C) haemolysis induced by 9, 13 or 18 μM PAL-CAR, respectively. Each value is the mean ± s.e.m. (n = 4 or 5). *$P < 0.05$, significantly different when compared with the value for the control group.

equivalent concentrations were approximately 10, 14 and 18 μM, respectively. These results suggest that PAL-CAR and PAL-LPC inflict damage to the erythrocyte membrane to a similar extent.

Fig. 2 shows the effects of dilazep and K-7259 on PAL-CAR-induced mild (n = 4 in each group), moderate (n = 5 in each group) and severe (n = 5 in each group) haemolysis. Dilazep significantly attenuated the mild, moderate and severe haemolysis induced by PAL-CAR at concentrations of 10 and 100 μM, although it did not attenuate the severe haemolysis induced by PAL-CAR at the lowest concentration (1 μM). The highest concentration of dilazep (1 mM), however, failed to attenuate PAL-CAR-induced haemolysis. K-7259 significantly attenuated mild, moderate and severe haemolysis induced by PAL-CAR at concentrations ranging from 1 μM to 1 mM, although the lowest concentration of K-7259 (1 μM) did not attenuate the severe haemolysis.

Fig. 3 shows the effects of dilazep and K-7259 on PAL-LPC-induced mild (n = 4 in each group), moderate (n = 5 in each group) and severe (n = 4 in each group) haemolysis. Dilazep significantly attenuated the mild, moderate and severe haemolysis induced by PAL-LPC at concentrations ranging from 1 to 100 μM, although the lowest concentration of dilazep (1 μM) did not attenuate the mild haemolysis. The highest concentration of dilazep (1 mM), however, failed to attenuate PAL-LPC-induced haemolysis. K-7259 attenuated the mild, moderate and severe haemolysis induced by PAL-LPC at concentrations ranging from 1 μM to 1 mM, although the effects of lower concentrations (1 and 10 μM) on the mild haemolysis were insignificant. These results suggest that both dilazep and K-7259 protect the erythrocyte membrane from

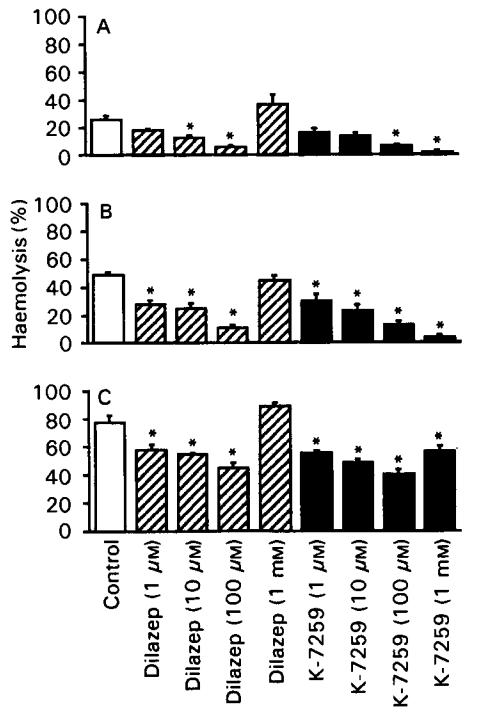


FIG. 3. Effects of dilazep and K-7259 on the mild (A), moderate (B) and severe (C) haemolysis induced by 10, 14 or 18 μM PAL-LPC, respectively. Each value is the mean ± s.e.m. (n = 4 or 5). *$P < 0.05$, significantly different when compared with the value for the control group.

damage induced by PAL-CAR or PAL-LPC whereas the protective effect of dilazep is nullified at high concentrations.

*Micelle formation*

Fig. 4 shows the effects of dilazep (100 $\mu$M) or K-7259 (100 $\mu$M) on micelle formation by PAL-CAR (n = 4 in each group) or PAL-LPC (n = 4 in each group). The fluorescence intensity increased markedly when concentrations of PAL-CAR and PAL-LPC were higher than 4 $\mu$M, suggesting that the critical micelle concentration (CMC) of PAL-CAR and PAL-LPC is approximately 4 $\mu$M. Dilazep and K-7259 did not modify the increase in fluorescence intensity induced by PAL-CAR or PAL-LPC, suggesting that neither dilazep nor K-7259 affects micelle formation by these compounds. These results indicate that the protective action of dilazep and K-7259 against the haemolysis induced by PAL-CAR and PAL-LPC is not a result of prevention of the formation of the these amphiphiles' micelles.

*Effects of dilazep and K-7259 on the hypotonic haemolysis*

In the preliminary experiment we found that the concentrations of NaCl in the buffer producing 25% (mild), 50% (moderate) and 75% (severe) haemolysis were about 63, 59 and 54 mM, respectively (data not shown). Fig. 5 shows the effects of dilazep and K-7259 on mild (n = 5 in each group), moderate (n = 5 in each group) and severe (n = 4 in each group) hypotonic haemolysis. Dilazep and K-7259 at concentrations of 1 and 10 $\mu$M had no effect on hypotonic haemolysis. At 100 $\mu$M, however, both dilazep and K-7259 significantly attenuated mild, moderate and severe hypotonic haemolysis. In contrast, dilazep and K-7259 significantly accelerated mild, moderate and severe hypotonic haemolysis at the highest concentration (1 mM), although the effect of K-7259 on severe haemolysis was not significant. Nevertheless, the highest concentration of both drugs (1 mM) did not produce haemolysis in the isotonic buffer (data not shown), indicating that neither dilazep nor K-7259 itself has a direct haemolytic action. These results suggest that dilazep and K-7259 have biphasic effects on hypotonic haemolysis and that the beneficial action of dilazep and K-7259 on the hypotonic haemolysis was less potent than that on the PAL-CAR- or PAL-LPC-induced haemolysis.

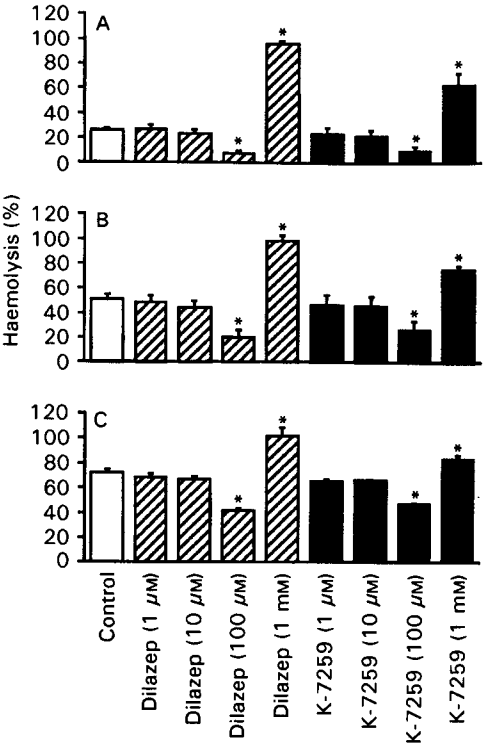


FIG. 5. Effect of dilazep or K-7259 on the mild (A), moderate (B) and severe (C) hypotonic haemolysis induced by 63, 59 or 54 mM NaCl, respectively. Each value is the mean ± s.e.m. (n = 4 or 5). *$P < 0.05$, significantly different compared with the value for the control group.

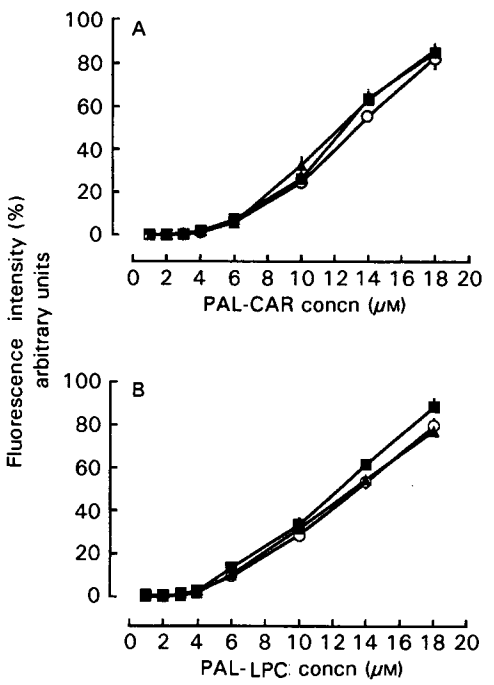


FIG. 4. Effects of dilazep (100 $\mu$M) or K-7259 (100 $\mu$M) on micelle formation by PAL-CAR or PAL-LPC. A. The fluorescence intensity obtained from 10 mM phosphate buffer (pH 7·4) containing PAL-CAR (○), PAL-CAR and dilazep (▲) or PAL-CAR and K-7259 (■). B. The fluorescence intensity obtained from 10 mM phosphate buffer (pH 7·4) containing PAL-LPC (○), PAL-LPC and dilazep (▲) or PAL-LPC and K-7259 (■). The fluorescence intensity obtained from the buffer containing PAL-CAR (20 $\mu$M) or PAL-LPC (20 $\mu$M) was taken as 100%; that obtained from the normal buffer (without PAL-CAR or PAL-LPC) was taken as 0%. Each value is the mean ± s.e.m. (n = 4).

## Discussion

Amphiphilic metabolites, including PAL-CAR and PAL-LPC, are known to exist in fluid as monomers at concentrations below the CMC and exist as both monomers and micelles at concentrations above the CMC (Yalkowsky & Zografi 1970; Utsumi et al 1978; Weltzien 1979; Bergmann et al 1981). In the current study, both PAL-CAR and PAL-LPC caused erythrocyte haemolysis (as evidenced by release of haemoglobin) at concentrations above the CMC (4 $\mu$M). This result is supported by previous findings that the concentration of PAL-CAR or PAL-LPC required to produce haemolysis is above the CMC (Utsumi et al 1978; Bergmann et al 1981; Piper et al 1984). According to an electron spin resonance study, PAL-

LPC monomer is incorporated into the phospholipid bilayers of the erythrocyte membrane, but does not cause haemolysis (Utsumi et al 1978). These findings suggest that both PAL-CAR and PAL-LPC cause haemolysis by formation of micelles. Dilazep and K-7259 attenuated significantly or not significantly the haemolysis induced by PAL-CAR or PAL-LPC at concentrations of 1 $\mu$M to 1 mM, except for the highest concentration of dilazep (1 mM). These results suggest that both dilazep and K-7259 protect the erythrocyte membrane from damage induced by PAL-CAR or PAL-LPC. It should be noted, however, that the protective effects of dilazep and K-7259 are not a result of inhibition of micelle formation, because neither dilazep (100 $\mu$M) nor K-7259 (100 $\mu$M) affected micelle formation by PAL-CAR or PAL-LPC.

By what mechanism, then, does dilazep or K-7259 protect the erythrocyte membrane from PAL-CAR- or PAL-LPC-induced damage? PAL-CAR and PAL-LPC inhibit $Na^+K^+$ adenosine triphosphatase in biological membranes, including the erythrocyte membrane, and hence would reduce the intracellular $Na^+$ concentration in the erythrocyte (Pitts & Okhuysen 1984; Tamura et al 1987). In addition, PAL-LPC increases $Na^+$ influx in erythrocytes through components pharmacologically similar to the $Na^+$ channel in excitable tissues (Bierbaum et al 1979). The increase in intracellular $Na^+$ would result in the osmotic imbalance between intra- and extracellular media that is responsible for membrane damage and hence haemolysis (Bierbaum et al 1979). To simulate the osmotic imbalance, erythrocytes were placed in the hypotonic solution and the effects of dilazep and K-7259 on hypotonic haemolysis were studied. The results indicated that the anti-haemolytic action of dilazep or K-7259 on hypotonic haemolysis was not observed at concentrations (1 or 10 $\mu$M) that inhibit PAL-CAR- or PAL-LPC-induced haemolysis. If the haemolytic action of PAL-CAR or PAL-LPC is a consequence of osmotic imbalance (intracellular osmotic pressure higher than extracellular osmotic pressure), dilazep or K-7259 would not inhibit the PAL-CAR- or PAL-LPC-induced haemolysis at the concentration (1 or 10 $\mu$M) that was ineffective at attenuating hypotonic haemolysis (intracellular osmotic pressure higher than extracellular osmotic pressure). Therefore, the action of dilazep and K-7259 at reducing PAL-CAR- or PAL-LPC-induced haemolysis might be because they prevent osmotic imbalance rather than protecting the erythrocyte membrane against damage induced by osmotic imbalance. Nevertheless, the detailed mechanisms of the anti-haemolytic action of dilazep and K-7259 are not clear, although it is possible that dilazep and K-7259 inhibit $Na^+$ influx in erythrocytes because dilazep has an $Na^+$-channel-blocking action in excitable tissues (Hashizume et al 1994).

Both dilazep and K-7259 accelerated hypotonic haemolysis at the highest concentration (1 mM), although they exerted anti-haemolytic action at 100 $\mu$M. Similar biphasic action on hypotonic haemolysis has been observed with propranolol (Surewicz et al 1980; Rogers et al 1986). These workers demonstrated that propranolol interacts with membrane protein or lipoprotein to attenuate hypotonic haemolysis, whereas at higher concentrations it partitions into membrane lipids to accelerate hypotonic haemolysis. By interaction with membrane proteins or lipids, therefore, dilazep and K-7259 might also result in biphasic action on hypotonic haemolysis. Nevertheless, it remains obscure why the protective effect of dilazep against the PAL-CAR- or PAL-LPC-induced haemolysis was diminished at the highest concentration (1 mM). Further studies are needed to determine detailed mechanisms of the action of dilazep and K-7259 on PAL-CAR- or PAL-LPC-induced damage.

In conclusion, dilazep and K-7259 protect the erythrocyte membrane from PAL-CAR- or PAL-LPC-induced haemolysis. The protective effects of dilazep and K-7259 are mediated by some mechanism other than prevention of micelle formation and protection of the erythrocyte membrane against osmotic imbalance.

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## References

Abe, H., Katada, K., Orita, M., Nishikibe, M. (1991) Effects of calcium antagonists on the erythrocyte membrane. J. Pharm. Pharmacol. 43: 22–26

Anderson, R., Ramafi, G., Theron, A. J. (1996) Membrane stabilizing, anti-oxidative interactions of propranolol and dexpropranolol with neutrophils. Biochem. Pharmacol. 52: 341–349

Bergmann, S. R., Ferguson, T. B. Jr, Sobel, B. E. (1981) Effects of amphiphiles on erythrocytes, coronary arteries, and perfused hearts. Am. J. Physiol. 240: H229–H237

Bierbaum, T. J., Bouma, S. R., Huestis, W. H. (1979) A mechanism of erythrocyte lysis by lysophosphatidylcholine. Biochim. Biophys. Acta 555: 102–110

Cho, K. S., Proulx, P. (1971) Studies on the mechanism of hemolysis by acyl carnitines, lysolecithins and acyl cholines. Biochim. Biophys. Acta 225: 214–223

Corr, P. B., Saffitz, J. E., Sobel, B. E. (1987) Lysophospholipids, long-chain acylcarnitines and membrane dysfunction in the ischaemic heart. Basic Res. Cardiol. 82 (Suppl 1): 199–208

Datorre, S. D., Creer, M. H., Pogwizd, S. M., Corr, P. B. (1991) Amphipathic lipid metabolites and their relation to arrhythmogenesis in the ischemic heart. J. Mol. Cell Cardiol. 23 (Suppl I): 11–22

Hara, A., Abiko, Y. (1996a) Protective effects of dilazep and its derivative, K-7259, on mechanical and metabolic derangements induced by hydrogen peroxide in the isolated perfused rat heart. J. Pharmacol. Exp. Ther. 277: 565–571

Hara, A., Abiko, Y. (1996b) Role of the sympathetic nervous system in the ischemic and reperfused heart. In: Karmazyn, M. (ed.) Myocardial Ischemia: Mechanisms, Reperfusion, Protection, Birkhäuser, Basel, pp 285–297

Hara, A., Akahira, M., Abiko, Y. (1995) Effect of K-7259, a novel derivative of dilazep, on cardiovascular actions of adenosine: comparison with dilazep. Arch. Int. Pharmacodyn. Ther. 330: 66–75

Hara, A., Hashizume, H., Abiko, Y. (1996) Dilazep and its derivative, K-7259, attenuate mechanical dysfunction induced by palmitoyl-L-carnitine in the isolated, perfused rat heart. J. Pharmacol. Exp. Ther. 279: 32–38

Hashizume, H., Akiyama, K., Abiko, Y. (1994) Effects of antiischemic drugs on veratridine-induced hypercontracture in rat cardiac myocytes. Eur. J. Pharmacol. 271: 1–8

Hoque, A. N. E., Hoque, N., Hashizume, H., Abiko, Y. (1995a) A study on dilazep: I. mechanism of anti-ischemic action of dilazep is not coronary vasodilation but decreased cardiac mechanical function in the isolated, working rat heart. Jpn J. Pharmacol. 67: 225–232

Hoque, A. N. E., Hoque, N., Hashizume, H., Abiko, Y. (1995b) Cardioprotective effect of K-7259, a new dilazep derivative, in the ischemic-reperfused isolated working rat hearts (abst). Jpn J. Pharmacol. 67: 121

Hoque, A. N. E., Hoque, N., Hashizume, H., Abiko, Y. (1995c) A

study on dilazep: II. Dilazep attenuates lysophosphatidylcholine-induced mechanical and metabolic derangements in the isolated, working rat heart. Jpn J. Pharmacol. 67: 233–241

Mustafa, S. J. (1979) Effects of coronary vasodilator drugs on the uptake and release of adenosine in cardiac cells. Biochem. Pharmacol. 28: 2617–2624

Neufeld, K. J., Lederman, C. L., Choy, P. C., Man, R. Y. K. (1985) The effect of lidocaine on lysophosphatidylcholine-induced cardiac arrhythmias and cellular disturbances. Can. J. Physiol. Pharmacol. 63: 804–808

Ortner, M. J., Sik, R. H., Chignell, C. F., Sokoloski, E. A. (1979) A nuclear magnetic resonance study of compound 48/80. Mol. Pharmacol. 15: 179–188

Piper, M. H., Sezer, O., Schwartz, P., Hütter, J. F., Schweickhardt, C., Spieckermann, P. G. (1984) Acyl-carnitine effects on isolated cardiac mitochondria and erythrocytes. Basic. Res. Cardiol. 79: 186–198

Pitts, B. J. R., Okhuysen, C. H. (1984) Effects of palmitoyl carnitine and LPC on cardiac sarcolemmal $Na^+K^+$-ATPase. Am. J. Physiol. 247: H840–H846

Rogers, J. A., Cheng, S., Betageri, G. V. (1986) Association and partitioning of propranolol in model and biological membranes. Biochem. Pharmacol. 35: 2259–2261

Sasaki, Y., Morita, T., Takeyama, S. (1984) Effects of diltiazem on the physicochemical properties of rat erythrocyte and liposome membrane: comparison with pentoxifylline and propranolol. Jpn J. Pharmacol. 34: 417–427

Sugiyama, S., Kondo, T., Ajioka, M., Hattori, M., Nagai, S., Ozawa, T. (1985) The effects of dilazep on reperfusion arrhythmias. Arzneim. Forsch. 35: 1802–1804

Surewicz, K. W., Fijalkowska, I., Leyko, W. (1980) The effect of propranolol on the osmotic fragility of red cells and liposomes and the influence of the drug on glycerol transport across the membrane of red cells. Biochem. Pharmacol. 30: 839–842

Tamura, M., Harris, T. M., Higashimori, K., Sweetman, B. J., Blair, I. A., Inagami, T. (1987) Lysophosphatidylcholine containing polyunsaturated fatty acids were found as $Na^+,K^+$-ATPase inhibitors in acutely volume-expanded hog. Biochemistry 26: 2797–2806

Utsumi, H., Inoue, K., Nojima, S., Kwan, T. (1978) Interaction of spin-labeled lysophosphatidylcholine with rabbit erythrocytes. Biochemistry 17: 1990–1996

Van Belle, H. (1993) Nucleoside transport inhibition: a therapeutic approach to cardioprotection via adenosine? Cardiovasc. Res. 27: 68–76

Weltzien, H. U. (1979) Cytolytic and membrane-perturbing properties of lysophosphatidylcholine. Biochim. Biophys. Acta. 559: 259–287

Yalkowski, S. H., Zografi, G. (1970) Potentiometric titration of monomeric and micellar acylcarnitines. J. Pharm. Sci. 59: 798–802