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Short communication

PK 11195 antagonizes the positive inotropic response of the isolated rat heart to diazepam but not the negative inotropic response

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Abstract

The influence of the ligand PK 11195 (1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide), antagonist of the peripheral-type benzodiazepine receptor, on the inotropic response of the perfused rat heart to diazepam (7-chloro-5-phenyl-methyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one) was studied. Diazepam induced a positive inotropic response which was preceded by a transient negative inotropic response. Concentrations of 10^{-7} M PK 11195 were ineffective, whereas concentrations of 10^{-6} and 10^{-5} M PK 11195 reduced the positive inotropic response significantly. At $5 \cdot 10^{-5}$ M PK 11195 the response was completely abolished. The negative inotropic response was not changed by either concentration of PK 11195 used. It is concluded that the positive inotropic response of the isolated rat heart to diazepam may well be mediated through peripheral-type benzodiazepine receptors; the negative inotropic response must be related to other (more complex) mechanisms.

Keywords: Benzodiazepine; Diazepam; Heart preparation, isolated; Inotropy; Peripheral-type benzodiazepine receptor antagonist

1. Introduction

Benzodiazepines are frequently prescribed for the treatment of anxiety in patients in whom the primary process is related to cardiovascular function after a recently experienced myocardial crisis, and their use in anaesthesia is gaining ground. Under each of these conditions heart trouble is involved or can be expected. Several studies have revealed that benzodiazepines may evoke changes in heart functioning (Akahane et al., 1987). More recently it was shown by Leeuwin et al. (1993) that benzodiazepines elicit both positive and negative inotropic responses in the isolated rat heart, depending on the concentration and the type of benzodiazepine derivative tested: diazepam (7-chloro-5-phenyl-methyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one) induces a biphasic response, whereas midazolam (8-chloro-6-(2-fluoro-phenyl)-1-methyl-4*H*-imidazo (1,5-*a*)-1,4-benzodiazepin) only evokes a positive inotropic response.

There is no consensus on the mechanism(s) underlying the inotropic responses. In the central nervous system benzodiazepines elicit their pharmacological actions through specific binding sites on GABA (γ-aminobutyric acid)-gated chloride channels. In addition to these central types of receptors, so-called peripheral-type benzodiazepine receptors have been identified by several authors in peripheral tissues (Verma and Snyder, 1989; Parola et al., 1993). Ligands such as Ro 5-4864 (7-chloro-5-(chlorophenyl)-1,3-dihydro-1-methyl-2*H*-1,4-benzodiazepin-2-one; 4′-chlorodiazepam), a structurally related benzodiazepine, and PK 11195 (1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide), structurally quite different from benzodiazepines, bind with high affinity to peripheral benzodiazepine-type receptors, but not to the central sites (Farges et al., 1993). PK 11195 is considered a peripheral benzodiazepine receptor antagonist on grounds of results of pharmacodynamic assays as performed by Le Fur et al. (1983).

This communication is the first report describing possible interference of PK 11195 with positive inotropic responses of the isolated heart to diazepam.

2. Materials and methods

Cpb:WU(WI) female Wistar rats, weighing 160–180 g, anaesthetized with pentobarbitone, and heparinized were used. Hearts were perfused in the Langendorff setup. After thoracotomy the aorta was cannulated rapidly in order to

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allow perfusion (retrograde). The heart was excised, placed in the experimental setup, instantly perfused with Tyrode's solution and gassed with 95% O_2 + 5% CO_2 at 37°C, under constant hydrostatic pressure of 60 cm Hg (perfusion rate 8 ml/min). Contractile force was measured as the left ventricular pressure. A latex balloon filled with water was inserted in the left ventricle measuring oscillations in force of heartbeat, and connected to a Gould Statham pressure transducer (P23ID). Ventricular pressure was recorded continuously on a Gould (2-channel) recorder, model 8188.2202.06. After equilibration and recording for 20 min (= control level in advance of the first administration), the experiment started with exposure of the heart preparation to a drug. This was followed by a subsequent recovery period and control registration, lasting 10 min, after the previous administration. Diazepam, $2 \cdot 10^{-5}$ to $6 \cdot 10^{-4}$ M, was administered (in 10 s) in subsequent increasing concentrations after each 10 min of control registration. Identical experiments were performed in the continuous presence in the perfusate of PK 11195 (10^{-7} to $5 \cdot 10^{-5}$ M). Inotropic responses – after each exposure of the preparation to a benzodiazepine – were expressed as percentage change of contractile force, measured when either maximum increase or depression was manifest, as compared to the force immediately before administration of a drug. Data collected before and after exposure to a benzodiazepine were analyzed statistically using Student's *t*-test. Each drug or combination of drugs was tested in 7 experiments. The drugs used were: diazepam (Valium, Roche Nederland, Mijdrecht, Netherlands) dissolved in propylene glycol; PK 11195 (Sanver Tech) dissolved in 96% ethanol (3.52 mg in 0.5 ml subsequently diluted with Tyrode's solution).

3. Results

Propylene glycol by itself administered to the perfusion fluid did not alter inotropy. PK 11195 in concentrations of 10^{-7} to $5 \cdot 10^{-5}$ M in otherwise unexposed preparations did not interfere with baseline contraction force. Fig. 1 shows a series of characteristic recordings of the inotropic response due to diazepam before and after exposure to PK 11195, 10^{-6}, 10^{-5} and $5 \cdot 10^{-5}$ M, and also after exposure to 10^{-5} M PK 11195 alone, the latter recording being illustrative for either concentration of the antagonist employed by itself. The biphasic nature of the inotropic response to diazepam – a transient decrease of the contractile force followed by a positive inotropic response abating at higher concentrations, was still manifest at 10^{-6} or 10^{-5} M PK 11195. At a concentration of $5 \cdot 10^{-5}$ M PK 11195 the positive inotropic response had completely vanished. The negative inotropic response however was not affected: it still occurred at all concentrations of PK 11195 employed. The baseline contraction force was 32.0 mm ± 4.1 (S.E.M.). After exposure to $1 \cdot 10^{-4}$ M diazepam the contraction force initially decreased to 15.6 ± 1.7 mm, subsequently followed by an increase of the force (positive inotropy) to 72.0 ± 4.1 mm. In the presence of 10^{-6} or 10^{-5} M PK 11195 the contractile force after exposure to diazepam $1 \cdot 10^{-4}$ M initially decreased to 17.0 ± 1.2 mm or 14.0 ± 1.1 mm respectively, preceding an increase of contractile force to 49.0 ± 3.0 mm or 42.0 ± 2.9 mm respectively. The quantitative results are summarized in Fig. 2. The shape of the curves showing the positive inotropic response in two steps is clearly recognized though parallel shifted at 10^{-7} or 10^{-6} M PK 11195, and to a lesser degree at 10^{-5} M as compared to that of diazepam

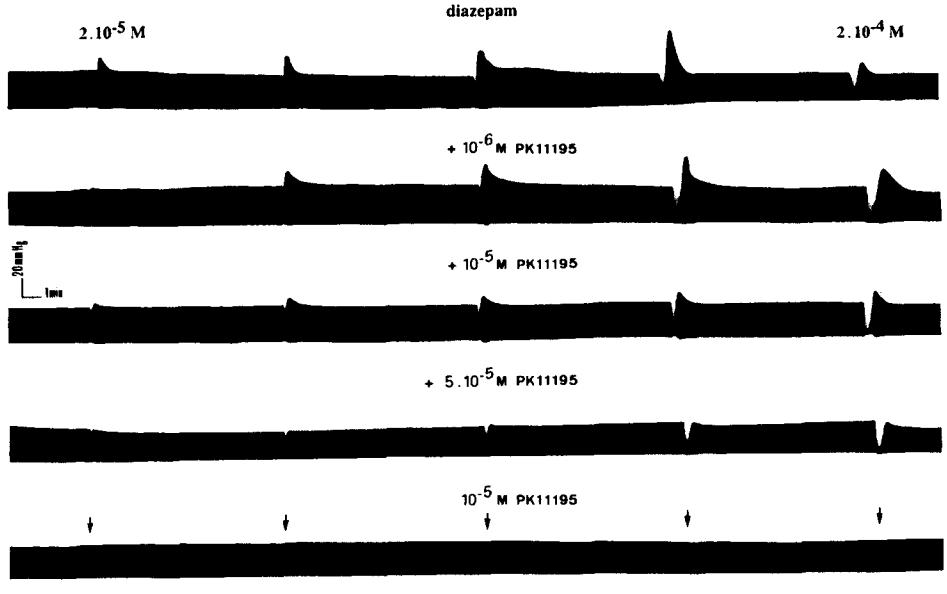

Fig. 1. Representative recordings of inotropic responses of the isolated rat heart to diazepam administered in stepwise increasing concentrations, alone and in the presence of 10^{-6}, 10^{-5} or $5 \cdot 10^{-5}$ M PK 11195. Owing to lack of space not all concentrations are mentioned in the figure.

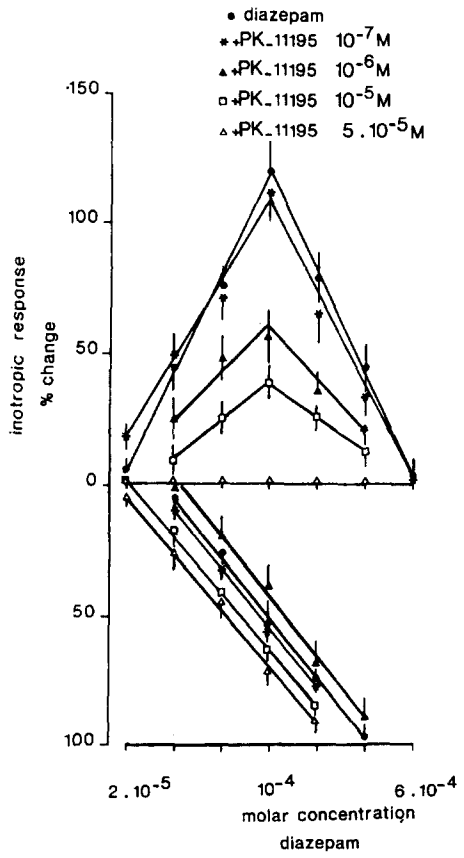

Fig. 2. Concentration-response curves of the inotropic responses of the isolated rat heart to diazepam alone and in the presence of PK 11195, shown as changes in force of contraction and compared to the contractile force in the absence of the drugs. Upper part of the abscissa: the effect of diazepam (means ± S.E.M.; $n = 7$) as positive inotropic response. Lower part of the abscissa: the effect of diazepam as a negative (means ± S.E.M.; $n = 7$) inotropic response. Owing to lack of space not all concentrations are mentioned in the figure.

alone. At the concentration of 10^{-7} M PK 11195 in the perfusate neither the positive nor the negative inotropic response was affected significantly ($P < 0.05$). The negative inotropic response to diazepam did not change significantly in the presence of either concentration of PK 11195 ($P > 0.05$). At $5 \cdot 10^{-5}$ M PK 11195 the curve of the negative inotropy seemed to be shifted to the left, indicating some potentiation, but the difference appeared to be statistically not significant ($P > 0.05$).

4. Discussion

We have tested the influence of the antagonist of the peripheral-type benzodiazepine receptor ligand PK 11195 on the inotropic responses of the perfused Langendorff rat heart to exposure to the benzodiazepine diazepam. PK 11195 when administered by itself did not affect the basal force of contraction; however, in the concentration range of 10^{-7} to 10^{-5} M the positive inotropic response to diazepam was significantly antagonized; at $5 \cdot 10^{-5}$ M PK 11195 this response was completely abolished. The negative inotropic response to diazepam remained unchanged, using the same concentrations of the agonist and antagonist. Higher concentrations of PK 11195 were not studied because of solubility problems.

Reports related to cardiac action of benzodiazepines are conflicting and confusing. Leeuwin et al. (1993) have shown that benzodiazepines may elicit both positive – in two steps – and negative inotropic responses in the isolated Langendorff rat heart. The quality and quantity of the responses depend on the type of benzodiazepine derivative involved and the concentration used (Leeuwin et al., 1993). The mechanism(s) underlying these actions is (are) obscure. As yet there is no satisfactory explanation available for the shift of the concentration-response curve downwards. Receptor desensitization may be involved; or two effects, opposing each other, may be involved, mediated by different mechanisms and alternately dominating each other.

So-called peripheral-type benzodiazepine receptors occurring in different organs of various species, including the heart (Charbonneau et al., 1986), to which (sub)classes of benzodiazepines can bind, have been recognized during the last decade. In view of the present study it is enticing to attribute the positive inotropic response of the heart to diazepam to binding at the peripheral-type of benzodiazepine receptor, this binding being blocked by PK 11195. The lack of sensitivity in our study of the negative inotropic action of diazepam to PK 11195 might imply that it originates at a site in the membrane other than that of the positive response, in this case the peripheral-type benzodiazepine receptor. Using Ro 5-4864, Holck and Osterrieder (1985) showed that in micromolar concentrations this benzodiazepine analogue may bind to other sites as well, which could result in triggering of pharmacological actions, among them possibly the negative inotropic action of diazepam, explaining the lack of efficacy of PK 11195 in preventing it. This argument would be in agreement with observations of Shany et al. (1994) who found a negative inotropic effect of Ro 5-4864 in human atrial muscle strips, which PK 11195 failed to antagonize.

Other mechanisms can be or have been postulated as well. Akahane et al. (1987) using the perfused canine atrium described a biphasic inotropic response – inhibition followed by stimulation – when diazepam was injected directly into the sinus node artery. Propranolol inhibited the positive inotropic response, whereas the duration of the negative inotropic response was prolonged. Imipramine in a dose which blocked the tyramine-induced positive inotropic response, augmented the positive inotropic response to diazepam, suggesting that diazepam induces direct negative inotropic effects, the mechanism of which remains to be defined. Since Ca^{2+}-entry blockers also modulate the inotropic responses to benzodiazepines (Leeuwin et al., 1994), it seems that Ca^{2+} currents are involved as well. Bender and Hertz (1985) presented evidence that the non-

neuronal diazepam binding site in glial cell cultures is associated with Ca^{2+} channels. Moreover, findings of Bolger et al. (1990) strongly suggest a functional association between the peripheral-type of benzodiazepine receptor and voltage-operated Ca^{2+} channels in the guinea pig and rat cardiovascular system. Likewise involvement of adenosine uptake mechanisms must be considered since it has been shown that benzodiazepines potentiate pharmacological responses to adenosine in cardiac muscles (Clanachan and Marshall, 1980). The possibility must be considered that adenosine receptor mechanisms are involved with the negative inotropic response to diazepam. It is well known that adenosine receptor agonists may evoke negative inotropic responses in the isolated heart, which are reversed by adenosine antagonists (Shryock et al., 1992; Mudumbi et al., 1993). At present effects of the latter on responses of the isolated heart to benzodiazepines are under investigation. As yet there are no indications of any interference.

In conclusion, the benzodiazepine diazepam induced a transient negative followed by a positive inotropic response in the perfused rat heart. The peripheral-type benzodiazepine receptor ligand PK 11195 abolished the positive and not the negative inotropic response. These findings may imply that the negative inotropic response originates at other (receptor) (sub)sites or that other mechanisms must be involved.

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