Diazepam Increases Calcium Sensitivity of the Skinned Cardiac Muscle Fiber in Guinea Pig

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Received May 26, 1999    Accepted July 8, 1999

ABSTRACT—Influences of diazepam, a benzodiazepine derivative, on the contractile response to calcium in skinned trabecular fibers of guinea pig heart were examined. Diazepam (100 μM) enhanced the contractile response of the skinned fiber to calcium and shifted the concentration-response curve to the left. The pCa50 values were 6.07±0.03 and 6.28±0.03 (P<0.05) in the absence and presence of diazepam, respectively. This result suggests that diazepam increases calcium sensitivity of contractile proteins in heart muscles.

Keywords: Calcium sensitivity, Diazepam, Skinned cardiac muscle

Diazepam, a benzodiazepine derivative, has an established role in human and veterinary anesthesia regimes. This drug is commonly used as a tranquilizer, muscle relaxant, and an anticonvulsant agent in clinical medicine (1). Following ingestion of therapeutic dosages of diazepam, virtually no cardiac effects are observed in otherwise healthy patients (2). However, an intravenous injection of 5 to 10 mg diazepam in humans may cause a decrease in ventricular force (1). A slight but significant decrease in heart rate was found after an intravenous administration of diazepam in the open-chest dog preparation (3). It has been suggested that diazepam could also affect cardiac contractility; however, the data and their interpretation were rather contradictory. For example, positive, negative, and biphasic inotropic actions or no effect of diazepam have been reported in different species of animals using various ranges of concentrations (4–7).

There has been no report to the best of our knowledge that showed diazepam’s influence on calcium sensitivity of contractile proteins. Therefore, the aim of the present paper was to test whether diazepam altered the calcium sensitivity in skinned trabecular fibers of guinea pig heart.

This study was done in compliance with the “Guiding Principles for the Care and Use of Laboratory Animals” approved by The Japanese Pharmacological Society.

Healthy guinea pigs (250–500 g) of both sexes were used. Skinned fibers were prepared from ventricular trabeculae of guinea pig heart using the method of Temma et al. (8). Briefly, the hearts of guinea pigs were excised under pentobarbital anesthesia and mounted in a Langendorff apparatus, perfused with N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid] (HEPES) buffered solution to wash them completely free of blood, and then trabeculae of the ventricle were obtained. Trabeculae of 100- to 200-μm width were cut to a length of 2–3 mm for experimental use. One end of the muscle fiber was fixed to a tungsten rod (300 μm in diameter) and the other to an L-shaped tungsten bar connected to an isometric transducer (TB-651T; Nihon Kohden, Tokyo). The muscle fiber preparations were set horizontally in an incubation solution (100 μl, 24°C) and a pen recorder (Wi621 G, Nihon Kohden) was used to continuously record the generated tension. Skinned muscle fibers that were exposed to a relatively high concentration of saponin (1 mg/ml) for 30 min in order to completely disrupt the functions of sarcoplasmic reticulum and mitochondria (9) and cell membranes were used in our experiments. In these skinned muscle fibers, contractions induced by a relatively high concentration of caffeine (20 mM) were abolished. It is necessary to disrupt the functions of sarcoplasmic reticulum and mitochondria, which store calcium, in order to study the calcium sensitivity of skinned fiber. Ten calcium-ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid (EGTA) buffer solutions with calcium concentration ranging from pCa 4.19 to 7.35 were prepared and applied sequentially to the skinned fibers. When the changes in contraction had stabilized, the skinned fiber was washed with a relaxing solution. The calcium concentration-response curves were obtained using a series of calcium-EGTA buffer solution (8).
HEPES buffered solution contained 105.5 mM NaCl, 5.6 mM KCl, 1.0 mM MgCl₂, 1.0 mM CaCl₂, 5.5 mM glucose and 3 mM HEPES (pH 7.4). The relaxing solution contained 20 mM 3-[N-morpholino]propanesulfonic acid (MOPS), 2 mM EGTA, 4.3 mM ATP-Na₂ and 5 mM Mg<sup>2+</sup>-methanesulfonate. Calcium-EGTA buffer solution: one solution containing 10 mM EGTA and 10 mM Ca<sup>2+</sup>, and another solution containing 10 mM EGTA were mixed up with varying portions of the solutions to prepare calcium concentrations ranging from pCa 4.19 to 7.33. In addition, both solutions contained 20 mM MOPS, 4.3 mM ATP-Na₂ and 5 mM Mg<sup>2+</sup>-methanesulfonate. Relaxing solution and calcium-EGTA buffer solution were adjusted to pH 7.00 and an ionic strength of 0.17 (24°C) using KOH and K<sup>+</sup>-methanesulfonate, respectively.

Diazepam (Yamanouchi Pharmaceuticals Co., Ltd., Tokyo) was dissolved in dimethyl sulfoxide (Wako Junyaku, Osaka). All other chemicals used were reagent grade.

Results are expressed as fraction of the increase in force of contraction produced by a maximum concentration of calcium. Values are presented in terms of the mean ± S.E.M. Student's t-test was used for statistical analysis of the data and considered significant at a P value less than 0.05.

A typical example of the contractile responses in skinned fiber preparations in absence (control) and presence of diazepam (100 μM) is shown in Fig. 1. It has been reported that this concentration of diazepam produced the maximal positive inotropic effect in a Langendorff heart preparation of the rat (7). An augmented contractile response of the skinned fiber to calcium was observed in the presence of diazepam. In these skinned muscle fiber preparations, an increase in calcium concentration in the incubation medium caused a typical sigmoidal concentration-response curve (Fig. 2). Diazepam shifted the concentration-response curve for calcium to the left. The calcium concentrations that caused half maximal contraction (pCa<sub>50</sub>) were calculated to be 6.07±0.03 (Hill coefficient, r=0.811) and 6.28±0.03 (P<0.05, r=0.901) in the control and diazepam (100 μM)-treated fiber, respectively.

To the best of our knowledge, this study is the first to demonstrate an increase in calcium sensitivity of contractile proteins in cardiac tissue by diazepam. Increasing calcium sensitivity of contractile proteins is important for positive inotropism. We have previously reported that diazepam potentiates the contractile effect of histamine through increasing cyclic AMP level in guinea pig heart (10). Another laboratory also reports that diazepam potentiates the positive inotropic effect induced by β-adrenoceptor agonists through enhancing the cyclic AMP production in rat ventricular strip (11). Thus, diazepam may also contribute to the positive inotropism through enhancement of cyclic AMP level induced by histamine and β-agonists.

However, some studies showed that diazepam itself possessed a negative inotropic effect in the heart. Daniell (3) reported that diazepam caused a decrease in contractile force of canine heart in a dose-dependent manner.

![Fig. 1. Representative recordings of calcium contraction of the ventricular trabecula skinned fiber preparation isolated from guinea pig heart in the absence (control) or presence of diazepam (100 μM). Increase in contractile response of the skinned fiber preparation to calcium was observed in the presence of diazepam.](image-url)
The negative inotropic effect of diazepam was also observed in rat right ventricular strips (12) and in the papillary muscle from guinea pig right ventricle (13). Another study has shown that diazepam elicits a biphasic response in the Langendorff preparation of rat heart, i.e., a transient negative inotropic action preceding a positive inotropic response (7, 14). The negative inotropic effect induced by diazepam was partly related to suppression of the transsarcolemmal calcium current in cardiac cells (13, 15).

As mentioned above, diazepam could affect cardiac contractility; however, the data and their interpretation were rather contradictory. The actual values of force of contraction probably represents the sum of the two effects of diazepam; i.e., inhibition of the calcium current and enhancement of the myofilament calcium sensitivity. The relative contribution of these effects to the inotropic response to diazepam may be dependent on the species and the cardiac preparation being studied. This could partly explain the apparent discrepant results so far reported.

In conclusion, diazepam in a concentration of 100 μM enhanced the contractile response of the skinned myocardial fiber to calcium. Further studies, including concentration-response relationships, are necessary to elucidate precise mechanism(s) of inotropic effects of diazepam on cardiac tissue.

Acknowledgments

The authors would like to thank Ahmmed Ally, M.D., Ph.D., Department of Pharmacology, University of New England, College of Osteopathic Medicine, Biddeford, ME, USA, for critically reading the manuscript.

REFERENCES