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Response of Isolated Human Ventricular Myocardium to Cyclic AMP and Its Dibutyryl Derivative*

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Summary. The contractile responses to c-AMP and DB-c-AMP were studied in isolated electrically stimulated human papillary muscle strips. C-AMP (1×10^{-4} to 1×10^{-3} M) had no effect on contractile force in all of 6 human papillary muscle preparations studied. In contrast, DB-c-AMP (10^{-4} to 5×10^{-3} M) produced a concentration-dependent and reversible positive inotropic effect which was associated by a decrease in time to peak force and in relaxation time and which was not inhibited by 10^{-6} M propranolol. The possibility of a clinical applicability of DB-c-AMP is discussed.

Key words: Isolated human ventricular myocardium, c-AMP, DB-c-AMP, Positive inotropic effect.

Die inotrope Wirkung von cyclischem AMP und seinem Dibutyrylderivat am isolierten menschlichen Ventrikelmyokard.

Zusammenfassung. Am isolierten menschlichen Ventrikelmyokard hatte c-AMP (10^{-4}—10^{-3} M) keinen Einfluß auf die Kontraktionskraft. Im Gegensatz dazu wirkte DB-c-AMP (10^{-4}—$5 \cdot 10^{-3}$ M) konzentrationsabhängig und reversibel positiv inotrop. Dieser Effekt ging einher mit einer Verkürzung von Anstiegzeit und Erschlaffungszeit der Kontraktion und wurde durch Vorbehandlung mit Propranolol nicht beeinflußt.

Schlüsselwörter: Isoliertes menschliches Ventrikelmyokard, c-AMP, DB-c-AMP, Positiv inotroper Effekt.

Several attempts have been made to mimic the positive inotropic effects of catecholamines with c-AMP or its butyrated derivatives. In cardiac preparations isolated from various laboratory animals, dibutyryl-c-AMP (DB-c-AMP) usually increased myocardial force [see 8 for ref.] whereas c-AMP itself was ineffective or negatively inotropic [4, 12, 1, 8]. With regard to the human heart, the effects of c-AMP and DB-c-AMP on myocardial mechanics, although possibly of clinical importance, are as yet not defined. The few in-vivo-data reported [5–7] are only partially conclusive and studies on isolated preparations are still lacking. Hence, the present study was designed to evaluate the effects of c-AMP and DB-c-AMP on mechanical behaviour of ventricular preparations isolated from human hearts.

Methods

Left ventricular papillary muscles were excised from 8 rheumatic heart disease patients during cardiac surgery at the time of mitral valve replacement. Patients ranged in age from 37 to 52 years (mean 46); 6 were male and 2 female. Six of these patients suffered from combined mitral valve lesions with predominant mitral regurgitation, one had pure mitral stenosis and one had pure mitral insufficiency. The patients were characterized by cardiac hypertrophy and chronic cardiac failure of clinical severity degree II–III. All patients had been treated with digoxin for several years and most of them received diuretics (thiazides, spironolactone) and antibiotics before being operated. General anesthesia was performed with neuroleptic-narcotic combinations (haloperidol, fentanyl and nitrous oxide).

Immediately after excision, the papillary muscles were placed in cold (4°C) Tyrode solution and delivered to the laboratory. The time between excision and the beginning of laboratory processing was never longer than 90 min. In order to minimize inadequate oxygenation of the central fibres, each native papillary muscle was splitted into thin preparations which yielded stable contractions for at least 5 hours. This dissection was performed in oxygenated Tyrode solution at room temperature and was carried out so that the fibres in the preparation ran parallel to the length of the strip. Muscle strips ($N = 35$) had a mean cross-sectional area of 1.22 ± 0.09 mm², their length was 6.01 ± 0.25 mm and their wet weight 8.77 ± 0.58 mg. Each strip was suspended in an organ bath containing 5 ml Tyrode solution (ref. 8; 1.8 mM Ca^{++}; pH 7.4; temperature 35°C) which was continuously gassed with 95% O_2 + 5% CO_2. Contractions were elicited by electrical stimulation (frequency 0.5 Hz) and were measured under isometrical conditions as described previously [8]. Under the conditions employed, liberation of tissue catecholamines was assumed to be minimal, if any, because the force of contraction was not decreased by propranolol (10^{-6} M). Drugs used included cyclic N^6-2′-O-dibutyryl-adenosine-3′,5′-monophosphate (DB-c-AMP; monosodium salt, Boehringer Mannheim), cyclic adenosine-3′, 5′-monophosphate (c-AMP; crystallized free acid, Boehringer Mannheim) and dl-propranolol-HCl (Rheinpharma Heidelberg). All drugs were freshly dissolved in Tyrode solution. Each concentration of each drug was tested in individual preparations.

Before exposure to the drug containing medium the preparations were allowed to equilibrate in Tyrode solution for at least 60 min. Contractile force was measured before (control or predrug values) and at the maximum of the drug effects. Isometric contraction curves were recorded at high paper speed (100 mm/sec) and were analyzed according to Reiter [10]. All experimental values are given as means ± S.E.M. Statistical comparions were performed by Stu-t-test. $P < 0.05$ was considered significant.

Results

Cyclic AMP (1×10^{-4} to 1×10^{-3} M) had no effect on the contractile behaviour of all of 6 human papillary muscle preparations studied, even when the preparations were exposed to this compound for 60 min.

In contrast to c-AMP, DB-c-AMP produced a concentration-dependent positive inotropic effect in this preparation; the threshold concentration was about 3×10^{-4} M; the peak concentration (5×10^{-3} M) increased contractile force to 266% of the control values (Fig. 1 B). The positive inotropic response of human papillary muscles to DB-c-AMP developed gradually (Fig. 1 A); it began in a concentration-dependent manner 16.8 (3×10^{-4} M) to 6.0 min (5×10^{-3} M) after addition of the drug and was at its peak within 54.0 (3×10^{-4} M) to 20.2 min (5×10^{-3} M). Once established, the positive inotropic effect of DB-c-AMP remained nearly constant within 120 min. DB-c-AMP did not cause any arrhythmias or contractures.

The effect of DB-c-AMP on contractile behaviour was found to be reversible. Predrug levels were reached after a 50 to 60 min wash in drug free solution. The effects could be repeated in the same preparation with virtually identical results.

* Some of the results have been presented at the 39th meeting of the Deutsche Gesellschaft für Kreislaufforschung, Bad Nauheim 1973.

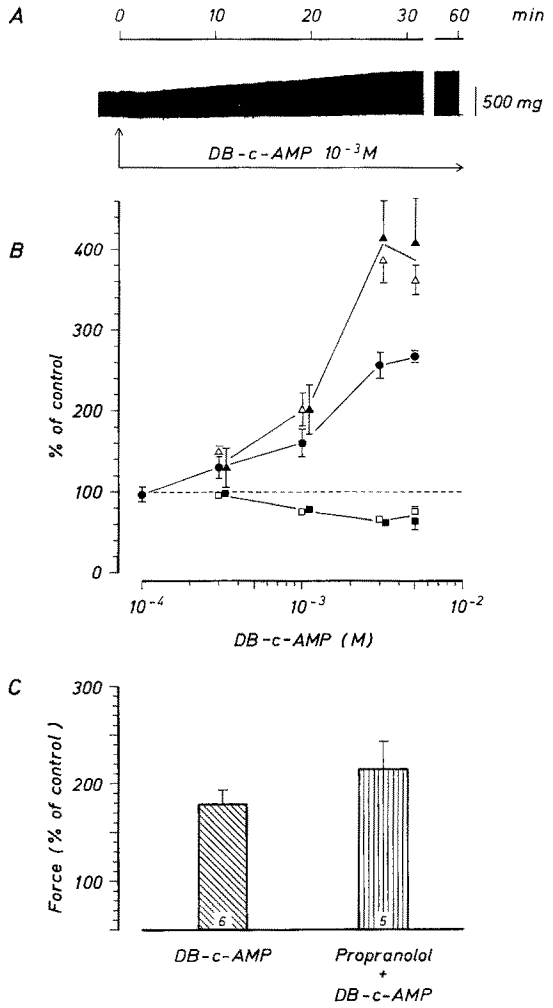

Fig. 1. Influence of DB-c-AMP on contractile behaviour of isolated human papillary muscle strips. A: Original tracing illustrating contractile force before and within 60 min after addition of 10^{-3} M DB-c-AMP. B: Influence of various concentrations of DB-c-AMP (abszissa; log. scale) on contractile force (●), mean rate of force development (▲) mean rate of relaxation (△), time to peak force (▪) and relaxation time (□). The effects of DB-c-AMP were evaluated at the maximum of the positive inotropic response and are given in % of the corresponding predrug values (ordinate). Each symbol represents the mean of 3–6 preparations. Predrug values: 0.114 ± 0.018 g/mm² (●, $N = 23$); 0.477 ± 0.089 g/mm² × sec (▲; $N = 17$); 0.272 ± 0.050 g/mm² × sec (△; $N = 17$); 223 ± 8.00 msec (▪; $N = 17$) and 385 ± 11.0 msec (□; $N = 17$). C: Positive inotropic effect of DB-c-AMP (10^{-3} M) in the absence (left column) and in the presence of 10^{-6} M propranolol (right column). Ordinates: Contractile force (macimum) in % of predrug values. Same preparations in both groups, N is given in the columns. Predrug values (g/mm²): 0.134 ± 0.015 (DB-c-AMP) and 0.109 ± 0.016 (Propranolol + DB-c-AMP). Propranolol was added 10 min prior to the addition of DB-c-AMP

As shown in Fig. 1B, the positive inotropic response to DB-c-AMP was accompanied by an increase in the mean rates of tension development and relaxation whereas the time to peak force and the relaxation time were decreased. These effects are similar to those observed with adrenaline [11].

The positive inotropic effect of DB-c-AMP (10^{-3} M) in human papillary muscles was not altered by 10^{-6} M pro pranolol (Fig. 1C).

One might argue that the preparations used were less suitable than papillary muscles from cats or other small animals. Although we cannot completely exclude that some of the preparations had a central core of tissue whose contractile performance was limited by inadequate oxygenation, it should be emphasized that, despite of these possible limitations, the positive inotropic response to DB-c-AMP was observed in all preparations studied.

Moreover, each muscle was capable of responding to this inotropic stimulus in a similar way regardless of the clinical and hemodynamic classification of the patient from whom it was derived.

Discussion

As compared to c-AMP, DB-c-AMP is thought to penetrate more readily into cells and to be less susceptible to enzymic degradation [9]. In contrast to c-AMP, DB-c-AMP markedly increased contractile force in human ventricular myocardium. The DB-c-AMP produced positive inotropic effect which is quite in accord with the results obtained in heart muscle preparations from other species [see 8 for ref.] was not inhibited by propranolol and is thus unlikely to be due to a release of endogenously stored catecholamines or to stimulation of adrenergic β-receptors.

Although DB-c-AMP, when given in doses sufficient to increase myocardial force, may have severe side effects such as abdominal pain, headache and myalgias [5] it possibly could be taken for therapeutic use. Since it apparently acts independeutly of β-adrenergic receptors, it may be useful in clinical states in which these receptors are blocked but a positive inotropic effect might be desired. Such patients include those with cardiogenic shock or congestive heart failure who have previously received β-adrenergic blocking drugs for the control of ventricular ectopic beats or to suppress digitalis-induced arrhythmias. It should be noted that DB-c-AMP caused no arrhythmias or contractures. Moreover, in contrast to glucagon [3] which was also found to act independently of adrenergic β-receptors [2], DB-c-AMP seems to be effective in human ventricular myocardium from patients with long-standing congestive heart failures.

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Buchbesprechungen

Moll, Werner: Kompendium der Rheumatologie. Ein Vademecum für Klinik und Praxis. 2., neu bearb. Aufl. Basel-München-Paris-London-New York-Sydney: S. Karger 1972. X, 288 S., 22 Abb. u. 18 Tab. DM 29,—.

Das jetzt in 2. Auflage erschienene „Kompendium der Rheumatologie" besticht nicht nur durch sein Format und seine Handlichkeit, sondern auch durch die Fülle und Prägnanz dessen, was auf 288 Seiten (mit einigen zusätzlichen für persönliche Notizen!) gebracht wird. Alle wichtigen entzündlichen und degenerativen Erkrankungen des Bewegungsapparates werden in übersichtlicher Weise abgehandelt, im allgemeinen nach dem Schema: Definition, Synonyma, Morbidität, pathologische Anatomie, klinische Erscheinungen, Laborbefunde, Diagnose und Differentialdiagnosen, Prognose, medikamentöse und physikalische Maßnahmen. Die zahlreichen offenen Probleme, die es gerade in der Rheumatologie noch gibt, sind in dem Kompendium nur angedeutet. Um so größer ist seine klinische Brauchbarkeit als zuverlässiger Ratgeber besonders in der Diagnostik und Differentialdiagnostik. Die allgemeinen Prinzipien der Therapie, z.B. der immunsuppressiven Therapie, sind relativ kurz gehalten. Doch verweist der Verfasser auf ein in Vorbereitung befindliches besonderes „Kompendium der Rheumatherapie"; auch sind bei den einzelnen Krankheiten genügend detaillierte Behandlungsvorschläge gemacht. Eine kurze Literaturübersicht am Schluß erleichtert die weitere Orientierung. Alles in allem: Ein für das Krankenbett überaus brauchbarer und handlicher Ratgeber, in dem bewußt auf eine Diskussion wissenschaftlicher Probleme verzichtet wird. Gross (Köln)

Psychosocial Aspects of Physical Illness. Edit.: Z. J. Lipowski. (Advances in Psychosom. Medicine. Edit.: J. Bastiaans H. Freyberger, L. Levi a.o. Vol. 8.) (Psychosoziale Aspekte körperlicher Erkrankungen). Basel-München-Paris-London-New York-Sydney: S. Karger 1972. XVI, 275 S., 17 Abb. u. 4 Tab. Geb. DM 108,—.

Die derzeitige Situation der Psychosomatik ist geprägt von einem Übergang der ausschließlich persönlichkeitsbezogenen Untersuchungen über Störungen im seelisch-körperlichen Bereich zu einer weiten Miterfassung und Einbeziehung der Umwelt des Kranken in den Forschungsbereich. Die noch vor wenigen Jahren vorherrschenden, wenn auch nicht unwidersprochen gebliebenen individuumbezogenen Interpretationen (z.B. von Alexander) treten mehr und mehr gegenüber einer breiter angelegten, insbesondere die psychosozialen Aspekte berücksichtigenden Forschung zurück. Es ist daher zu begrüßen, wenn der Herausgeber durch die sinnvolle Zusammenfassung der Arbeiten von 13 für die Entwicklung richtungweisenden Autoren eine momentane Bestandsaufnahme vornimmt. — In drei Hauptabschnitte (Psychosocial Determinants of Illness Onset, Psychological Responses to Illness: Their Determinants and Modes, The Patient and His Environment) gliedern sich die Beiträge. Originell erscheint eine Kommentierung am Schluß der Abschnitte I und II sowie III (Robert H. Ebert, Boston bzw. W. B. Spaulding, Hamilton). — Nicht ganz ließen sich einzelne Überschneidungen bei der vielseitigen Bearbeitung des Stoffes vermeiden. In manchen Kapiteln wäre der interessierte, fachkundige Leser für eine detaillierte Angabe von Forschungsergebnissen dankbar, dagegen würde er gern auf allgemeingehaltene und daher sich wiederholende Ausführungen zum Thema verzichten. — Im ganzen gesehen fördert diese Darstellung die Einbeziehung neuer Denkansätze in der psychosomatischen Forschung, die auch heute noch mancherorts einer gewissen Stereotypie unterworfen ist. — Der im Umgang mit englischer Fachliteratur kundige Leser wird dem Herausgeber dieses neuen Buches „Advances in Psychosomatic Medicine" dankbar sein.

Kleinsorge (Mannheim/Heidelberg)

Ergebnisse der Physiologie, biologischen Chemie und experimentellen Pharmakologie/Reviews of Physiology, Biochemistry and Experimental Pharmacology. Hrsg.: R. H. Adrian, E. Helmreich, H. Holzer, R. Jung, K. Kramer, O. Krayer, F. Lynen, P. A. Miescher, H. Rasmussen, A. E. Renold, U. Trendelenburg, W. Vogt u. H. H. Weber. Vol. 64. Berlin-Heidelberg-New York: Springer 1972. 342 S. u. 63 Abb. Geb. DM 98,—.

Im 64. Band der Ergebnisse der Physiologie wurden von G. Moruzzi, Pisa, und von M. Jouvet, Lyon, die Neurophysiologie und die Neurochemie des Schlaf- und Wachzustandes dargestellt. Moruzzi, selbst einer der erfolgreichsten Wissenschaftler auf dem Gebiet der Forschung über die neuronalen Mechanismen des Schlaf-Wachcyclus, stellt in den Mittelpunkt seiner Abhandlung die experimentell induzierten Modifikationen des Schlaf-Wachcyclus und weniger die Phänomenologie des Schlafes. Diese Orientierung am Experiment erscheint z. Zt. auch die einzige erfolgreiche Grundlage einer Analyse dieser komplexen Phänomenologie, da bereits Grundbegriffe wie Schlaf- und Wachzentren oder Aktivierungs- und Deaktivierungszentren allein durch Reiz- und Ausschaltversuche im Gehirn erkannt wurden. Moruzzis Darstellung und Versuch einer Synthese mit Beobachtungen am Wachtier bis hin zum Instinktverhalten und zur biologischen Rhythmik ist einmalig souverän und wohl z. Zt. von keinem anderen Autor in solch kompetenter Weise zu erwarten. — Als Partner zur neuro-funktionellen Analyse von Moruzzi gesellt sich in