Effect of Caffeine on Adenosine-Induced Heart Block in Guinea-Pigs

DRURY and SZENT-GYORGYI[1] were first to demonstrate that adenosine produces transient A-V heart block in guinea-pigs. The adenosine-induced heart block resembled electrocardiographically that produced by acetylcholine. The cholinergic mediation of the adenosine effect appeared, however, unlikely: atropine at doses which completely abolished acetylcholine-induced heart block, had no effect against adenosine[2,3]. The interest in cardiac actions of adenosine was renewed following recent hypothesis that adenosine is formed during myocardial hypoxia and induced vasodilation of coronary resistance vessels[4,5].

Various drugs were shown to either prolong or reduce the adenosine-induced heart block. Cardiac glycosides increased the duration of adenosine-induced heart block[6,7]. This effect was attributed not to the direct action of digitalis on the conduction system but to the inhibition of adenosine deaminase by digitalis[8]. The antagonism of adenosine-induced A-V heart block in guinea-pigs by caffeine was suggested by observations of THER et al.[3] and MCNAY[9]. According to MCNAY, 30 mg of caffeine i.v. per guinea-pig antagonized adenosine-induced cardiac asystole. Our studies were concerned with evaluation of the effect of caffeine on the adenosine-induced heart block; we demonstrated activity of caffeine at considerably lower doses, and dependency of the effect on the dose of caffeine as well as on the dose of adenosine.

Male guinea-pigs of 250–450 g body weight were anesthetized with sodium pentobarbital, 40 mg/kg i.p.; the jugular vein was cannulated and the electrocardiogram (Lead II) was recorded with a Sanborn 100 Viso one-channel recorder. Adenosine and caffeine were dissolved in 0.9% NaCl solution and administered i.v. in volumes not exceeding 1 ml/kg. Each animal received adenosine at 3 dose levels: 0.25, 0.5, and 1.0 mg/kg i.v. There was a 2 min interval between injections. The same series of injections was repeated 3 times at 5 min intervals. The second administration of adenosine, 0.25 mg/kg, produced heart block of longer duration than the first. There was no significant difference between the effects of the second and third doses of adenosine, and the response to the same dose of adenosine remained relatively stable during the subsequent hour. The duration of adenosine-induced heart block was dependent on the dose of adenosine (Table I).

Caffeine was administered at the following doses: 1.25, 2.5, 5.0, 10.0, 20.0, or 40.0 mg/kg i.v. within 2 min after the third series of adenosine injections. The administration of adenosine was repeated within 3 min and at 10, 20, 30, 45, and 60 min after caffeine. Caffeine reduced the duration of the adenosine-induced A-V heart block; it was considerably more effective against the smaller than against the larger doses of adenosine (Table II). The protective effect of caffeine usually reached its maximum within 3 min after its administration. The duration of caffeine action was dependent on the dose of caffeine; at 10 mg/kg it exceeded 60 min.

Sympathomimetics and particularly isoproterenol are commonly used in the treatment of the A-V heart block[10]. The cardiac adrenergic effects are thought to be mediated by activation of glycogen phosphorylase through stimulation of adenyl cyclase and resulting increase in 3′, 5′ cy clic AMP[11]. As an inhibitor of phosphodiesterase[12], caffeine can also be expected to increase the intracellular levels of 3′, 5′ cyclic AMP. The possibility was, therefore, considered that antagonism of adenosine-induced heart block is a consequence of inhibition of phosphodiesterase. Drugs known to affect phosphodiesterase were therefore investigated for possible effect on adenosine-induced heart block. Imidazole[12] and nicotinic acid[13] were reported to stimulate and diazoxide[14] to inhibit phosphodiesterase. Exogenous 3′, 5′ cyclic AMP produces, according to recent findings, cardiovascular and metabolic effects in animals and man[15,16]. In our experiments, in 3 out of 3 guinea-pigs, imidazole, 10 or 40 mg/kg i.v., had no effect on adenosine-induced A-V heart block. Nicotinic acid, 40 or

Table I. Duration of adenosine-induced A-V heart block in guinea-pigs

Dose of adenosine mg/kg i.v.	Duration of heart block, sec First series	Second series	Third series
0.25	3.4 ± 1.0	8.1 ± 0.9	8.8 ± 1.1
0.5	12.8 ± 1.7	11.7 ± 1.0	11.4 ± 0.7
1.0	20.1 ± 1.7	20.4 ± 1.1	21.1 ± 1.1

Average values for 10 animals ± standard errors.

Table II. Effect of caffeine on adenosine-induced A-V heart block in guinea-pigs

Dose of caffeine mg/kg i.v.	No. of animals	% reduction in duration of heart block produced by adenosine at mg/kg i.v. 0.25	0.5	1.0
1.25	5	45 ± 15	13 ± 4	
2.5	5	54 ± 7	25 ± 7	5 ± 3
5.0	7	96 ± 3	45 ± 6	22 ± 6
10.0	6	100 ± 0	68 ± 8	38 ± 7
20.0	5		100 ± 0	72 ± 5
40.0	8	100 ± 0	100 ± 0	82 ± 5

Average values ± standard errors. Maximal effect within 60 min after caffeine administration.

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80 mg/kg i.v., prolonged adenosine-induced A-V heart block in only 1 out of 6 animals and had no significant effect in 5 others. Diazoxide, 33 and 66 mg/kg i.v. had no significant effect on adenosine-induced heart block in 2 out of 2 experiments. 3',5' cyclic AMP, 8–20 mg/kg i.v., did not reduce adenosine-induced A-V heart block in 2 out of 2 guinea-pigs. These findings do not support the hypothesis that the protective effect of caffeine against adenosine induced A-V heart block is the result of inhibition of phosphodiesterase.

An alternative hypothesis involves the effect of caffeine on the intracellular Ca^{++}. Caffeine was demonstrated to inhibit the reaccumulation and to stimulate the release of Ca^{++} in mitochondria of the cardiac muscle of the toad[17]. Elevation of Ca^{++} concentration in isolated Purkinje fibers was recently demonstrated to hasten depolarization[18]. This effect can be associated with a shortening of the functional refractory period and can conceivably antagonize A-V heart block. Adenosine was shown to reduce the exchange of Ca^{++} associated with contractions of isolated guinea-pig atria[19]. It was also suggested that caffeine and adenosine have opposite effects on the binding of Ca^{++} at the critical site from which Ca^{++} is mobilized during contractions[20]. The antagonism of adenosine-induced heart block by caffeine may, therefore, involve an increase in the local concentration of the free Ca^{++}[21].

Zusammenfassung. Adenosin (0,25 mg/kg i.v. und mehr) ruft beim Meerschweinchen einen vorübergehenden atrioventrikulären Herzblock hervor. Coffein hat in Dosen von 1,25 mg/kg i.v. und mehr eine antagonistische Wirkung. Es wird angenommen, dass die Coffein-Wirkung auf der Beeinflussung des intrazellulären Ca^{++} Haushalts beruht.

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Geschwindigkeit der selektiven Akkumulation von Mercurascan-203 im geschädigten Muskel und im ischämischen Myokard

In den Quecksilber-II-Derivaten von Fluorescein[1,2] wurde eine neue Reihe von Stoffen gefunden, die die Fähigkeit besitzen, sich selektiv im ischämisch veränderten oder anders geschädigten Myokard und quergestreiften Muskel zu akkumulieren. Diese Eigenschaft kann zur Diagnostik ischämischer Veränderungen des Myokards mit Hilfe der Szintigraphie unter Verwendung von mit Quecksilberisotopen markierten Stoffen ausgenützt werden (Hg^{203} oder Hg^{197})[3–5]. In unseren früheren Arbeiten prüften wir die Akkumulation dieser Stoffe 24 h post injectionem. Nach diesem Intervall entsteht im ischämisch veränderten Muskel des Versuchstieres eine leicht darstellbare heisse Stelle. Das positive Szintigramm ist bedingt durch die gesteigerte Akkumulationsintensität im geschädigten Muskel und durch niedrige Hintergrundwerte ausserhalb der Ischämiezone. Die niedrigen Radioaktivitätswerte im gesunden Muskel und im Blut ergeben einen günstigen Index RI (Verhältnis der Radioaktivität von 1 g des geschädigten Muskels – gesunder Muskel) und RII (Verhältnis der Radioaktivität von 1 g des geschädigten Muskels – 1 ml Blut).

Tabelle

1. Hydroxymercurifluorescein-^{197}Hg bzw. ^{203}Hg (I)
2. Hydroxymercuri-2,7-dibromfluorescein-^{197}Hg bzw. ^{203}Hg (II)
3. Hydroxymercuri-2,7-dijodfluorescein-^{197}Hg bzw. ^{203}Hg (III)
4. Hydroxymercuri-4,5-dibromfluorescein-^{197}Hg bzw. ^{203}Hg (IV)
5. Bis-(hydroxymercuri)-fluorescein-^{197}Hg bzw. ^{203}Hg (V)

Die Bedeutung dieser Radioisotopendiagnostik wäre um so grösser je eher eine positive Szintigraphie nach dem Auftreten des Myokardinfarktes und nach der Applikation des Präparates entstehen würde. Wir untersuchten daher, wie schnell sich der verwendete Stoff im geschädigten Muskel akkumuliert, wie schnell die Ausscheidung aus dem Blut erfolgt und wie schnell ein positives Szintigramm am Ischämieherd des Myokards entsteht.

Als Detektor verwendeten wir radioaktives Hydroxymercurifluorescein (Hg^{203}), welches in der Gruppe der geprüften Quecksilber-II-Derivate des Fluorescein (Ta-

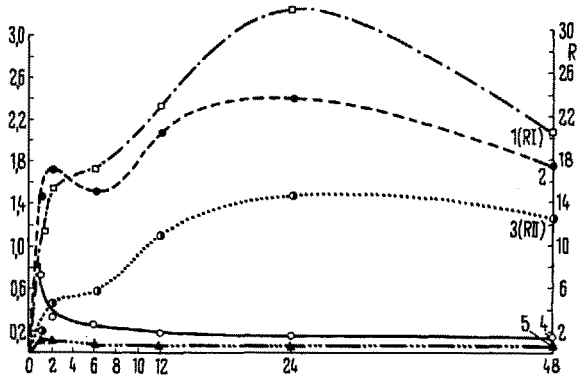

Fig. 1. Radioaktivitätswerte im geschädigten Muskel (Kurve 2), im Blut (Kurve 4) und gesunden Muskel (Kurve 5), ausgedrückt in % der gesamten verabreichten Aktivität per 1 g Gewebe und 1 ml Blut. Verschiedene Intervalle nach Mercurascan-203-Verabreichung (1047,800 imp/Ratte). Kurve 1: Index RI (Verhältnis geschädigter Muskel-gesunder Muskel). Kurve 3: Index RII (Verhältnis geschädigter Muskel–Blut). Auf der Senkrechten links Prozentsatz der gesamten verabreichten Aktivität, auf der Senkrechten rechts Werte für Index RI und RII. Auf der Waagrechten die Zeitspanne nach Mercurascan-203-Verabreichung in h.