Evidence for Glucagon-Releasing Activity of Vasoactive Adenosine Analogues in the Conscious Dog

W. Schütz, G. Raberger, and O. Kraupp
Pharmakologisches Institut der Universität Wien, Währinger Strasse 13a, A-1090 Wien, Austria

Summary. An investigation was carried out in conscious dogs concerning the effects of three adenosine derivatives substituted at the 5'- (744-96) or 2'-, 3'-, and 5'-positions (744-98, 744-99), with pronounced and long-lasting coronary dilator activity, on glucagon release. All three compounds (10 µg/kg i.v.) induced a sustained increase in plasma glucose and a decrease in plasma FFA concentration; concomitantly, plasma glucagon levels rose 2-3 fold. Changes in plasma insulin concentration were relatively small and of no statistical significance. A simultaneous fall in arterial blood pressure was also observed. A lowering of blood pressure of similar magnitude by sodium nitroprusside infusion in control experiments failed to show any significant effect on plasma glucagon level. These results point to a specific effect of vasoactive adenosine derivatives on glucagon release.

Key words: Adenosine analogues — Conscious dogs — Glucagon release — Insulin release.

Introduction

Recently published studies (Raberger et al., 1978; Schütz et al., 1978a) report pronounced metabolic actions of substance 744-98, a ribose-substituted adenosine derivative with long-lasting coronary dilator activity (Fig.1), both in the anaesthetized and in the conscious dog. The observed metabolic changes closely resembled those induced by exogenous glucagon in the dog: hyperglycaemia, a decrease in plasma free fatty acid (FFA) levels (Sokal et al., 1966; Trading et al., 1969; Whitty et al., 1969; Altszuler and Morrison, 1971) and hypokalaemia (Pettit et al., 1977). In this respect, Weir et al. (1975) reported of a glucagon-releasing activity of adenosine, ADP, and 5'-AMP in the isolated perfused rat pancreas. However, the effect of 744-98 on plasma FFA might rather be attributable to antilipolytic activity, a property of adenosine and related compounds (Dole, 1961; Ebert and Schwabe, 1973; Schillinger and Loge, 1974).

The aim of the present study was to investigate whether highly vasoactive adenosine derivatives also possess glucagon-releasing activity in vivo. Three structurally similar adenosine derivatives, all substituted at the ribose moiety and characterized by marked and long-lasting coronary dilator effects (Raberger et al., 1977), were used (Fig.1): the precursor substance, adenosine-5'- (N-ethylcarboxamide) (744-96), the most potent adenosine derivative on coronary vessels...
hitherto investigated; 2',3'-di-O-nitro-adenosine-5'-(N-ethylcarboxamidne) (744-99) and 2',3'-methoxymethyl-iden-adenosine-5'-(N-ethylcarboxamidne) (744-98), compounds with a delayed onset and prolonged duration of action as compared with 744-96. Studies were performed in conscious dogs with simultaneous measurement of the arterial levels of glucagon and insulin, glucose, FFA, and glycerol, and of haemodynamic parameters (arterial blood pressure and heart rate). These adenosine derivatives all produced a sustained fall in blood pressure. Control experiments were performed on dogs receiving sodium nitroprusside in order to eliminate a possible influence of a decrease in blood pressure. Control experiments were performed in conscious mongrel dogs of either sex, 17–26 kg in weight, which had been starved for 24 h. Each animal had been trained prior to the experiment to stand quietly in a rack and was required to remain standing for the entire experimental period. A Teflon catheter was inserted percutaneously into the femoral artery under local anaesthesia and connected to a Statham transducer for electromanometric pressure measurement. The arterial blood pressure and heart rate, integrated from the pulse pressure curve, were continuously registered on a Beckman RM dynograph. Blood samples were taken via the arterial cannula used for pressure recording for determination of glucagon, insulin, glucose, and FFA.

Three adenosine derivatives, 744-96, 744-98, and 744-99 (Fig. 1) were injected intravenously at a dosage of 10 µg/kg, 6 animals being used to test each compound. Since the purpose of this study was to measure only the maximum effects of each compound, experiments were terminated at the end of 1 h in the case of 744-96 and after 2 h in the case of 744-98 and 744-99 although the actions observed lasted for more than double the chosen times (Raberger et al., 1978). Four dogs, serving as controls, received sodium nitroprusside instead of an adenosine analogue in form of a 30-min infusion (10–15 µg/kg/min i.v.). Haemodynamic and metabolic measurements were carried out as described above.

**Assays.** The plasma glucagon concentration was determined by radioimmunoassay according to Faloona and Unger (1974), using the antiserum 30 K, which is highly specific for pancreatic glucagon. Plasma insulin concentration was determined by means of a commercial radioimmunoassay kit (The Radiochemical Centre, Amersham).

Plasma levels of glucose (Bergmeyer et al., 1970) and, in some experiments, glycerol (Eggesen and Kuhlmann, 1970) were analyzed enzymatically. Plasma FFA were determined according to Dole and Meinertz (1960).

**Materials.** The ribose-substituted adenosine derivatives 744-96, 744-98, and 744-99 were kindly donated by Byk Gulden Lomberg Chemische Fabrik GmbH, Konstanz. Sodium nitroprusside (Nipride®) was a gift from Hoffmann-La Roche & Co. AG, Basel.

The glucagon antiserum 30 K was purchased from the Southwestern Medical School, Texas University, Dallas; 123I-glucagon from Behringwerke AG, Marburg (Lahn); and the glucagon standard (porcine glucagon — behaving immunologically similar to canine pancreatic glucagon [Samols et al., 1966; Faloona and Unger, 1974]) from Novo Industri, Copenhagen. Enzymes for substrate analysis were obtained from Boehringer Mannheim and all other analytical grade reagents from Merck, Darmstadt.

**Statistics.** The statistical significance of changes induced by the adenosine analogues was assessed by the t-test for paired data.

**Results**

**Haemodynamic Effects**

As can be seen from Fig. 2, each of the adenosine analogues, administered at a dosage of 10 µg/kg i.v., induced a highly significant decrease in arterial blood pressure. 744-96 has the most pronounced vasodepressor effect at this dosage. This compound was shown by Raberger et al. (1977) to be characterized by the highest coronary vasodilator activity amongst 23 adenosine derivatives. In contrast to the baroreceptor-mediated increase in heart rate in the 744-98- and 744-99-treated groups, the apparently most potent compound, 744-96 (see Raberger et al., 1977), led even to a slight initial decrease in heart rate, probably because the adenosine-like direct negative chronotropic action (Drury and Szent-Gyorgyi, 1929; Schöndorf et al., 1969) predominates at this relatively high dosage of 744-96.

**Effects on Plasma Substrates and Hormone Levels**

The effects of 744-96 (10 µg/kg i.v.) on the arterial levels of glucose, FFA, glucagon, and insulin over the period of 1 h are depicted in Fig. 3. A pronounced rise in glucose and a fall in FFA levels were observed, maximum changes occurring 15 min following administration of the compound. The plasma glucagon concentration increased markedly from 133 ± 24 pg/ml (mean ± S.E.M.) to a maximum of 378 ± 68 pg/ml within 5 min. Values of glucose, FFA, and glucagon did not return to the pre-injection levels by the end of the 1-h observation period. In spite of the pronounced increase in plasma glucose and glucagon concentrations, there was only a slight but not significant rise in plasma insulin.

The effects of 744-98 and 744-99 (10 µg/kg i.v.) on the arterial levels of glucose, FFA, glycerol (measured only for 744-99), glucagon, and insulin are depicted in Fig. 4. At this dosage, the metabolic, like the haemodynamic effects observed with the 2',3',5'-substituted adenosine derivatives, were of a lesser magnitude than with the 5'-substituted parent substance, 744-96, and, moreover, the increase in glucose and decrease in FFA and glycerol levels were delayed. Plasma glucagon concentration rose from 76 ± 12 pg/ml to a maximum