Biochemical Pharmacology, Vol. 30, No. 12, pp. 1407–1414, 1981.
Printed in Great Britain.

0006-2952/81/121407-08 $02.00/0

EFFECTS OF GLUCAGON ON ^{45}Ca OUTFLOW EXCHANGE IN THE ISOLATED PERFUSED RAT HEART

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(*Received* 16 *August* 1979; *accepted* 5 *November* 1980)

Abstract—The effects of glucagon on cellular calcium distribution in cardiac muscle were studied using an isolated, spontaneously-beating, perfused rat heart preparation and a ^{45}Ca outflow exchange technique. The rate of loss of ^{45}Ca from the cellular space (intracellular calcium and calcium bound to extracellular sites) was determined, using [^{14}C]sucrose to estimate the rate of loss of ^{45}Ca from the extracellular space. The data were analysed by an iterative non-linear, least squares curve-fitting procedure. The simplest system which was found to be consistent with ^{45}Ca outflow exchange data obtained for control and glucagon-treated hearts is one in which three kinetically-distinct compartments of exchangeable calcium are associated with the cellular space. For control hearts, the fractional transfer rates (rate constants) for the outflow of calcium from compartments 1–3 were 0.61, 0.14 and 0.022 min^{-1}, respectively. Measurement of the amounts of ^{45}Ca present in subcellular fractions of perfused hearts indicated that compartment 3 represents calcium present in intracellular stores, including mitochondria and possibly the sarcoplasmic reticulum. The predominant effects of glucagon were to increase the fractional transfer rate for calcium outflow from compartment 1, and the quantity of exchangeable calcium in compartment 3.

The positive inotropic effects of glucagon and cyclic AMP on the heart have been shown to be associated with a change in the movement of calcium ions, detected using ^{45}Ca exchange techniques, in cardiac muscle preparations [1, 2]. However, little is known about the cellular location of the compartments of exchangeable calcium which are affected by these agents. Although it has clearly been shown that cyclic AMP modifies calcium transport in isolated sarcoplasmic reticulum of cardiac muscle (reviewed in [3]), the consequences of this change for the distribution of calcium in cardiac muscle cells or tissue have not been investigated in detail.

It has recently been reported that exposure of the heart to glucagon results in a stimulation of mitochondrial respiration [4]. Furthermore, studies on the liver, conducted in this laboratory, have shown that one of the actions of glucagon and dibutyryl cyclic AMP on this tissue is to modify the transport of calcium and phosphate ions and stimulate respiration in mitochondria subsequently isolated from the tissue [5, 6]. In view of these effects of glucagon or cyclic AMP on the sarcoplasmic reticulum [3] and mitochondria [4–6], it was of interest to investigate the effect of this agent on the intracellular distribution of calcium in intact cardiac muscle.

A ^{45}Ca outflow exchange technique, applied to the isolated spontaneously-beating perfused heart, was chosen for the present studies. The results indicate that under the conditions tested, the major effects of glucagon on intracellular calcium distribution are to increase the fractional transfer rate of exchangeable calcium present in a kinetically-distinct compartment with a short turnover time, and to increase the quantity of exchangeable calcium present in an intracellular compartment which includes the mitochondria.

MATERIALS AND METHODS

Heart perfusions. The hearts of male hooded-Wistar rats (Institute of Medical and Veterinary Science, Adelaide) of 220–320 g weight were perfused by the Langendorff method through a cannula inserted in the aorta. The perfusion medium (pH 7.4) [7] was composed of 118 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM phosphate, 25 mM $NaHCO_3$, 0.002% (w/v) phenol red, 11 mM glucose, 1.3 mM $CaCl_2$ and other additions as indicated, and was equilibrated with O_2:CO_2 (95:5). Preparation of the isolated perfused heart and its attachment to the perfusion apparatus [8] were conducted as described by Clark *et al.* [7]. The beating heart, maintained at a temperature of 37°, was suspended in air in a water-jacketed chamber from which the perfusate was collected or recirculated as indicated. The perfusion medium was continuously equilibrated [9] with O_2:CO_2 (95:5); the perfusion pressure was 50 cm H_2O and the flow rate 1.5–2.5 ml/min per g wet wt tissue.

^{45}Ca *outflow exchange*. Perfusate from the first 5 min of perfusion was discarded. The heart was then perfused with recirculated medium (about 80 ml) for 5 min before the addition of ^{45}Ca (0.6 MBq) to the reservoir of medium. Samples of the medium were removed for estimation of the concentration of ^{45}Ca. After the heart had been loaded with ^{45}Ca for a period of 60 min, the polyethylene cannula to the aorta was clamped, a second reservoir of fresh medium (previously equilibrated with O_2:CO_2 (95:5) at 37°), which did not contain ^{45}Ca, was connected to the inflow cannula, the external surface of the heart was briefly washed with fresh medium and the clamp on the inflow cannula released. This operation took about 15–20 sec. The perfusate was collected

in glass tubes at 0.5 or 1 min intervals using a fraction collector. The weight of perfusate in each tube was measured, and a sample removed for estimation of the ^{45}Ca content by liquid scintillation [5].

At the end of the perfusion, the heart was removed from the apparatus, weighed and homogenised in 10 ml of fresh perfusion medium for 2 min with an Ultra-Turrax TP 18-10N homogeniser (Janke and Kunkel K-G. IKA-Werk Breisgau). The amount of ^{45}Ca present in samples of the tissue, dissolved in Soluene-350 (Packard Instrument Co. Inc., Downers Grove, IL) was then measured.

When present, glucagon (2×10^{-7} M final concentration) was added to the reservoir of perfusion medium 15 or 30 min before the change from ^{45}Ca to ^{40}Ca was made. Glucagon, at the same concentration, was present in the fresh medium used for ^{45}Ca outflow.

Analysis of ^{45}Ca outflow exchange data. The simplest exponential equation which adequately describes the ^{45}Ca outflow exchange data was found to contain three exponential terms (see Results). This equation is consistent with the presence of three compartments of exchangeable calcium associated with the heart [10] from which ^{45}Ca is lost to the perfusion medium in exchange with ^{40}Ca. This ^{45}Ca does not return to the heart since the system is an "open" one. One arrangement of the compartments of exchangeable calcium in this three compartment open system is represented in Scheme 1 (see Results). The equation which relates q_4, the quantity of ^{45}Ca (per cent of initial dose of ^{45}Ca) present in the heart to the time elapsed after initiation of ^{45}Ca outflow exchange, t (min), is

$$q_4 = q_{41}\, e^{-\lambda_1 t} + q_{42}\, e^{-\lambda_2 t} + q_{43}\, e^{-\lambda_3 t}$$

where q_{jn} (per cent of initial dose of ^{45}Ca) and λ_n are constants [10]. Differentiation of this equation with respect to time gives the following expression for the rate (per cent of initial dose·min^{-1}) at which ^{45}Ca is lost from the heart

$$\frac{dq_4}{dt} = q_{41}\,\lambda_1\, e^{-\lambda_1 t} + q_{42}\,\lambda_2\, e^{-\lambda_2 t} + q_{43}\,\lambda_3\, e^{-\lambda_3 t} \quad (1)$$

The total amount of ^{45}Ca in each fraction of perfusate plus that which remained in the heart at the end of the experiment was used to determine the total amount of ^{45}Ca associated with the heart at the beginning of ^{45}Ca outflow (the initial dose of ^{45}Ca). The amount of ^{45}Ca eluted per minute in each fraction was then expressed as a percentage of the initial dose euluted from the heart (dq/dt) and plotted as a function of the time of outflow, t.

The minimum number of kinetic compartments required to explain the ^{45}Ca outflow exchange data was obtained by determining the minimum number of terms in the sum of exponentials equations (compare with equation (1)) required to adequately fit the data. Fitting of exponential equations and the compartment configuration (Scheme 1) was performed by an iterative, non-linear, least squares procedure in which, except where indicated otherwise, the standard deviations of the data were used for calculation of statistical weights [11]. The SAAM 27 version of the computer programme developed by Berman [11] was employed. Error variances for the estimated parameters were obtained from the normal equations matrix and the residual variance of the data [12].

The goodness of fit of exponential equations to the experimental data was assessed by the criteria that (i) standard deviations of the estimated constants were low; (ii) the calculated values of dq/dt did not deviate systematically from the observed data and (iii) a minimum weighted sum of squares was obtained. The attainment of a minimum sum of squares during sequential addition of terms to the sums of exponentials equations was assessed using the F test [12]. The degree of significance in the difference between the values of a given constant for control and glucagon-treated hearts was determined using the t test as described by Boxenbaum *et al.* [12].

Analysis of the ^{45}Ca outflow exchange data in the form dq/dt was found to introduce less systematic error to estimates of values for the constants of the exponential equations than expression of the data

Table 1. Distribution of ^{45}Ca in the perfused heart after equilibration with 1.3 mM $^{45}CaCl_2$*

Parameter measured	Experimental condition	
	Control	Glucagon
Wet weight of heart (g)	1.59 ± 0.06	1.53 ± 0.10
Volume of extracellular fluid (ml per heart)	0.99 ± 0.11	1.21 ± 0.12
^{45}Ca concentration in perfusion medium at equilibrium (10^{-5} × cpm per ml)	3.43 ± 0.22	2.70 ± 0.18
Total ^{45}Ca associated with heart (10^{-5} × cpm per heart)	5.25 ± 0.48	4.32 ± 0.66
^{45}Ca in extracellular space† (10^{-5} × cpm per heart)	3.40 ± 0.44	3.27 ± 0.39
(Percentage of total ^{45}Ca associated with heart)	65 ± 10	76 ± 15
^{45}Ca in cellular space† (10^{-5} × cpm per heart)	1.85 ± 0.65	1.05 ± 0.77
(Percentage of total ^{45}Ca associated with heart)	35 ± 13	24 ± 18

* Heart perfusions in the presence or absence of glucagon (2×10^{-7} M) were performed as described in Materials and Methods. The volume of extracellular fluid, the total amount of ^{45}Ca associated with the heart and the amounts of ^{45}Ca in the extracellular and cellular spaces at equilibrium were determined as described in Materials and Methods. The values are the means ± S.E.M. ($n = 6$ or 7).

† Values for S.E.M. were calculated as described by Uhr and Morrison [16].

in terms of q as a function of time. Plots of q (estimated by calculating the difference between the total amount of ^{45}Ca eluted at a given time, t, and the total amount of ^{45}Ca initially present in the tissue) as a function of time are often employed in the analysis of ^{45}Ca outflow exchange from tissues [13]. However, in the experiments reported here, it was found that estimates of q are subject to a large error when about 90 per cent of the ^{45}Ca has been lost from the heart.

During the changeover from perfusion in the presence of ^{45}Ca to that in the presence of ^{40}Ca, the cannula leading to the heart was briefly clamped in order to reduce the amount of ^{45}Ca-labelled perfusate associated with the heart at the beginning of the ^{45}Ca outflow exchange period. A test of whether the interruption in the flow of medium to the heart effects the ^{45}Ca outflow exchange curve was made by conducting similar experiments with control hearts in which the cannula leading to the aorta was not clamped during the change from ^{45}Ca to ^{40}Ca. Visual comparison of the ^{45}Ca outflow exchange curves obtained by the two methods revealed no significant differences between them. When equation (1) was fitted to the data obtained for hearts which had not been clamped ($n = 4$), the values of the constants were not significantly different (as judged by the t-test [12]) from those obtained for a fit of equation (1) to the data for control hearts (results not shown). Therefore, it is concluded that the temporary interruption of the flow of medium through the heart does not alter the transport and/or distribution of calcium in the heart within the level of detection used in the present study.

Contribution to ^{45}Ca outflow by ^{45}Ca initially present in the extracellular fluid. Hearts were perfused as described for ^{45}Ca outflow exchange experiments (1.3 mM $CaCl_2$) but without ^{45}Ca present. At 50 min after the start of the perfusion, 0.2 MBq of [^{14}C]sucrose (or [^{3}H]inulin in some experiments) was added to the perfusion medium (about 80 ml). Samples of the recirculated medium were removed and the concentration of [^{14}C]sucrose determined. After 15 min, the perfusion medium was changed to fresh medium (no [^{14}C]sucrose present). Collection of the perfusate, measurement of the [^{14}C]sucrose content of each fraction and of the heart, and expression and analysis of the results were performed as described for the ^{45}Ca outflow exchange experiments. The volume of extracellular fluid associated with the heart (Table 1), which includes about 0.15 ml of medium in the cannula, was estimated from the measured total amount of [^{14}C]sucrose associated with the heart after equilibration with [^{14}C]sucrose, and the concentration of [^{14}C]sucrose in the medium.

When present, glucagon (2×10^{-7} M final concentration) was added at 15 or 30 min before perfusion of the heart in the absence of [^{14}C]sucrose was begun, and was present at this same concentration in the [^{14}C]sucrose-free medium.

The rate of loss of ^{45}Ca from the extracellular space was estimated from the amount of ^{45}Ca associated with the extracellular space (Table 1) and the equations $dq/dt = 151e^{-2.4t} + 14e^{-0.49t}$ % initial dose·min^{-1} and $dq/dt = 85e^{-1.8t} + 18e^{-0.55t}$ % initial dose·min^{-1} for the rate of loss of [^{14}C]sucrose from control hearts and hearts perfused in the presence of glucagon, respectively. The full equations for the rate of loss of [^{14}C]sucrose (Table 4) were obtained from analysis of the data of Fig. 2C and D. In making a correction for the contribution of the extracellular space, the third exponential term was omitted because the very low fractional transfer rate (approximately equal to λ_3) of about 0.08 min^{-1} suggested that the loss of [^{14}C]sucrose in this phase does not represent loss from the extracellular space.

It can be seen (Table 1) that after the loading period, ^{45}Ca located in the extracellular space accounts for about 70 per cent of the total ^{45}Ca associated with the heart. The rate of loss of ^{45}Ca from the cellular space at any given time was estimated by subtracting the rate of loss from the extracellular space from the rate of loss of ^{45}Ca from the whole heart. The difference was then expressed as a percentage of the initial dose of ^{45}Ca present in the cellular space.

Subcellular fractionation. The heart was removed from the perfusion apparatus, placed in about 6 ml of ice-cold isolation medium, which consisted of 250 mM sucrose, 10 mM Tris–HCl and 10 mM EDTA, pH 7.5, cut into small pieces and homogenised for 3–4 sec with an Ultra-Turrax homogeniser. EDTA was included in the isolation medium in order to reduce possible redistribution of Ca between subcellular components during homogenisation and fractionation [5, 14]. The homogenate was separated into four fractions by differential centrifugation as described by Harigaya and Schwartz [15]. Each fraction was homogenised further to give a smooth suspension, and the amount of ^{45}Ca present in samples of the homogenate determined after treatment with Soluene-350 (Packard Instrument Co., Inc., Downers Grove, IL).

Materials. $^{45}CaCl_2$, [U-^{14}C]sucrose and [^{3}H]inulin were obtained from the Radiochemical Centre, Amersham, Bucks., U.K. and glucagon from the Sigma Chemical Company, MO. All other reagents were of the highest grade available.

RESULTS

The effect of glucagon on the frequency of contraction of the spontaneously-beating perfused rat heart is shown in Fig. 1B. After an initial increase in frequency, a new plateau is reached. This is significantly higher than the frequency of contraction of control hearts (Fig. 1A), and declines slightly as the perfusion continues.

Equation (1) was found to give a good fit to data for the loss of ^{45}Ca from the perfused heart in outflow exchange experiments conducted in the absence or presence of glucagon (Fig. 2A and B, and Table 2 (total ^{45}Ca associated with the heart)). The numerical values and S.D. of the constants obtained for a fit of equation (1) to the data are given in Table 2. Equations which contain only two exponential terms gave poor fits to the data (results not shown), and the addition of a fourth exponential term did not improve the goodness of fit. Thus it is concluded that equation (1) is the simplest equation which is consistent with the data. When compared with the data for control hearts, glucagon was found to cause

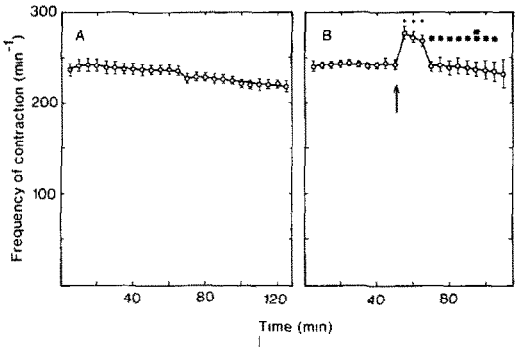
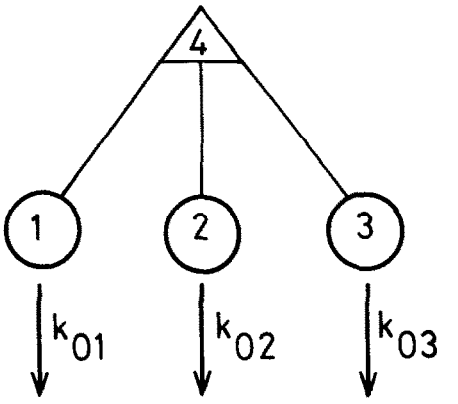


Fig. 1. Frequency of contraction of spontaneously-beating perfused hearts as a function of time in the absence (A) and presence (B) of glucagon during ^{45}Ca outflow exchange experiments. Heart perfusions were conducted as described in Materials and Methods. Glucagon (2×10^{-7} M) was added at the time indicated by the arrow. The change from perfusion in the presence of ^{45}Ca to perfusion in the absence of ^{45}Ca was made at 65 min. The values are the mean ± S.E.M. of 7 (control) and 4 (glucagon) experiments. The degrees of significance, determined using Student's *t*-test for unpaired samples, are: * $P < 0.05$; ** $P < 0.02$; and † $P < 0.002$.

increases in the constants ($q_{41}\lambda_1$) and λ_1, and a decrease in λ_3 (Table 2).

Since the simplest equation which is consistent with the ^{45}Ca outflow exchange data for both control and glucagon-treated hearts contains three exponential terms, it can be concluded that a system composed of three kinetically-distinct compartments of exchangeable calcium associated with the heart and its extracellular space is also consistent with the data [10]. One possible arrangement of these compartments, a parallel configuration, is represented in Scheme 1. For this configuration, values of the quantity of exchangeable Ca in compartment j, Q_j; the fractional transfer rate (rate constant) for calcium outflow, k_{0j}; and the flux of Ca across the compartment boundary, R_{0j}; can be estimated directly from the values obtained for the constants of equation (1) [13]. The numerical values of the constants and method of calculation are given in Table 3. The



Scheme 1. A schematic representation of the parallel configuration of the three compartments of exchangeable Ca detected by analysis of ^{45}Ca outflow exchange curves. The three kinetically-distinct compartments of exchangeable Ca associated with the heart are represented by ①. At any given time after initiation of ^{45}Ca outflow exchange, the sum of the quantity of ^{45}Ca present in each of these compartments ($q_1 + q_2 + q_3$) is represented by △ and is equivalent to the measured amount of ^{45}Ca associated with the heart, $q_4 (= q_1 + q_2 + q_3)$. The fractional transfer rate (rate constant) for the transport of ^{45}Ca into the perfusate from compartment j is represented by k_{0j}. The quantity of exchangeable Ca in each compartment is represented by Q_j (nmol·mg^{-1} (wet wt)) and the amount of Ca transported into the perfusate from compartment j per unit time (Ca flux), by R_{0j} (nmol·min^{-1}·mg^{-1} (wet wt)).

effect of glucagon is to increase Q_1 and Q_3, the quantity of exchangeable calcium in compartments 1 and 3, respectively; to increase k_{01} and decrease k_{03}; and to increase R_{01}, the flux across the boundary between compartment 1 and the medium.

The parallel configuration (Scheme 1) is only one of a number of configuartions of three compartments of exchangeable calcium which give ^{45}Ca outflow exchange curves described by equation (1). Since the numerical values of λ_1, λ_2, and λ_3 differ by 5–10-fold, and the quantity of exchangeable calcium in the perfusion medium is very much larger than that present in the heart, the numerical values

Table 2. Values and S.D. of the constants of the exponential equations which describe the rate of loss of ^{45}Ca from hearts perfused in the presence of 1.3 mM $^{40}CaCl_2$, and in the presence and absence of glucagon*

Source of ^{45}Ca	Perfusion conditions	$q_{41}\lambda_1$ (%·min^{-1})	λ_1 (min^{-1})	$q_{42}\lambda_2$ (%·min^{-1})	λ_2 (min^{-1})	$q_{43}\lambda_3$ (%·min^{-1})	λ_3 (min^{-1})
Total ^{45}Ca associated with the heart	Control	65 ± 6	1.07 ± 0.07	7.7 ± 0.9	0.22 ± 0.02	0.60 ± 0.05	0.033 ± 0.002
	Glucagon	93 ± 6‡	1.28 ± 0.06†	5.9 ± 0.6	0.21 ± 0.02	0.66 ± 0.07	0.027 ± 0.002†
^{45}Ca associated with the cellular space	Control	51 ± 2	0.61 ± 0.05	9.9 ± 2.0	0.14 ± 0.03	0.96 ± 0.5	0.022 ± 0.010
	Glucagon	183 ± 8¶	1.57 ± 0.06¶	11.7 ± 0.8	0.15 ± 0.02	2.5 ± 0.5†	0.025 ± 0.004

* Equation (1) was fitted to the data of Figures 2A, 3A (total ^{45}Ca associated with the heart) and 4 (^{45}Ca associated with the cellular space) as described in Materials and Methods. In the case of the data for ^{45}Ca associated with the cellular space (Fig. 4), the standard deviation of each value of dq/dt was estimated using the expression S.D. = $\sqrt{(dq/dt)}$. "^{45}Ca associated with the cellular space" is defined as total ^{45}Ca associated with the heart minus the ^{45}Ca in the sucrose space, and was calculated as described in Materials and Methods. The values of the constants q_{jn} (%·min^{-1}) are expressed as a percentage of the initial dose of ^{45}Ca associated with the heart (^{45}Ca associated with the heart) or the cellular space (^{45}Ca associated with the cellular space). The degrees of significance, P, determined using the *t*-test [12], are † $P < 0.05$; ‡ $P < 0.01$ and ¶ $P < 0.001$.

Table 3. Values and S.D. of the constants obtained for a fit of a three-compartment parallel configuration (Scheme 1) to the ^{45}Ca outflow exchange data for control and glucagon-treated perfused hearts*

	Control hearts	Glucagon-treated hearts
Quantity of exchangeable Ca (nmoles/mg wet wt)		
Q_1	0.76 ± 0.03	0.99 ± 0.02‡
Q_2	0.43 ± 0.02	0.38 ± 0.01
Q_3	0.23 ± 0.01	0.33 ± 0.01‡
Fractional transfer rate (min^{-1})		
k_{01}	1.07 ± 0.07	1.28 ± 0.06†
k_{02}	0.22 ± 0.02	0.21 ± 0.02
k_{03}	0.033 ± 0.002	0.027 ± 0.002†
Flux of Ca across compartment boundary ($nmol \cdot min^{-1}$/mg wet wt)		
R_{01}	0.81 ± 0.07	1.26 ± 0.07‡
R_{02}	0.10 ± 0.01	0.08 ± 0.01
R_{03}	0.007 ± 0.001	0.009 ± 0.001

* The values of Q_j ($= q_{jn}\lambda_n/\lambda_n$), k_{0j} ($= \lambda_n$) and R_{0j} ($= Q_j k_{0j}$) were calculated from the values of the constants given in Table 2 (total ^{45}Ca associated with the heart), the specific activity of the ^{45}Ca at the end of the ^{45}Ca loading period (calculated from the data of Table 1) and the weights of the hearts (Table 1) as described in Materials and Methods. The degrees of significance, P, determined using the *t*-test [12], are † $P < 0.05$ and ‡ $P < 0.001$.

obtained for Q_j, k_{0j} and R_{0j} for the parallel configuration (Scheme 1) are also approximate estimates [13] of the values of these parameters for compartments 1, 2 and 3 if, as is likely, they are arranged in other configurations.

In order to estimate the contribution made to ^{45}Ca outflow exchange from the heart by ^{45}Ca initially present in the extracellular space, the rate of loss of [^{14}C]sucrose from hearts equilibrated with this isotope was measured under the same experimental conditions. Equation (1) was found to be the simplest equation which is consistent with the data obtained for control (Fig. 2C) and glucagon-treated (Fig. 2D) hearts. When the contribution of the loss of ^{45}Ca from the extracellular space to the ^{45}Ca outflow exchange curves (Fig. 2A and B) was subtracted (as described in Materials and Methods), the curves shown in Fig. 3 were obtained for plots of ^{45}Ca outflow exchange from the cellular space as a function of time. The simplest equation which gave a good fit to the data for control and glucagon-treated hearts was found to be equation (1) (Fig. 3 and Table 2 (^{45}Ca associated with the cellular space)). Glucagon was found to increase the values of the constants ($q_4\lambda_1$), λ_1 and ($q_{43}\lambda_3$).

A system in which three compartments of exchangeable calcium are associated with the cellular space is also consistent with the data obtained for ^{45}Ca outflow from the cellular space of control and glucagon-treated hearts. Since considerable error is associated with estimation of the amount of ^{45}Ca in the cellular space at the beginning of the outflow period (Table 1), it was not possible to determine numerical values of Q_j and R_{0j} for these data. However, values of k_{0j} were determined ($k_{01} = \lambda_1$; $k_{02} = \lambda_2$; $k_{03} = \lambda_3$), making the assumptions described above [13]. For control and glucagon-treated hearts, respectively, these values are: k_{01}, 0.61 ± 0.05 and 1.57 ± 0.06 ($P < 0.001$); k_{02}, 0.14 ± 0.03 and 0.15 ± 0.02; and k_{03}, 0.022 ± 0.010 and 0.025 ± 0.004.

The distribution of ^{45}Ca between subcellular fractions isolated from homogenates of control perfused hearts was determined at 5 and 40 min after the initiation of ^{45}Ca outflow exchange (Table 5). Over this time period the smallest change in ^{45}Ca content was in fractions 2 (enriched with mitochondria) and 3 (enriched with microsomes derived from the sarcoplasmic reticulum).

DISCUSSION

The experiments reported were designed to investigate the effects of glucagon on calcium movement in the heart under conditions where the amounts of calcium associated with myocardial cells and their intracellular components (averaged over many beats) do not change markedly. It is considered that this is a reasonable assumption since (a) the frequency of contraction of the hearts changed only slightly during the measurement of ^{45}Ca outflow, (b) a period of 60 min was allowed for equilibration of the hearts with ^{45}Ca, and (c) hearts were exposed to glucagon for 15 or 30 min before initiation of ^{45}Ca

Table 4. Values and S.D. of the constants of the exponential equations which describe the rate of loss of [^{14}C]sucrose from hearts perfused in the presence and absence of glucagon*

Perfusion conditions	$q_{41}\lambda_1$ ($\%\cdot min^{-1}$)	λ_1 (min^{-1})	$q_{42}\lambda_2$ ($\%\cdot min^{-1}$)	λ_2 (min^{-1})	$q_{43}\lambda_3$ ($\%\cdot min^{-1}$)	λ_3 (min^{-1})
Control	151 ± 26	2.38 ± 0.36	14.3 ± 3.6	0.49 ± 0.07	1.48 ± 0.26	0.074 ± 0.009
Glucagon	85 ± 18†	1.80 ± 0.36	17.8 ± 7.9	0.55 ± 0.12	3.00 ± 0.34‡	0.097 ± 0.005

* Equation (1) was fitted to the data of Figures 2B and 3B as described in Materials and Methods. The values of the constants q_{jn} ($\%\cdot min^{-1}$) are expressed as a percentage of the initial dose of [^{14}C]sucrose associated with the heart. The degrees of significance, P, determined using the *t*-test [12], are † $P < 0.05$ and ‡ $P < 0.001$.

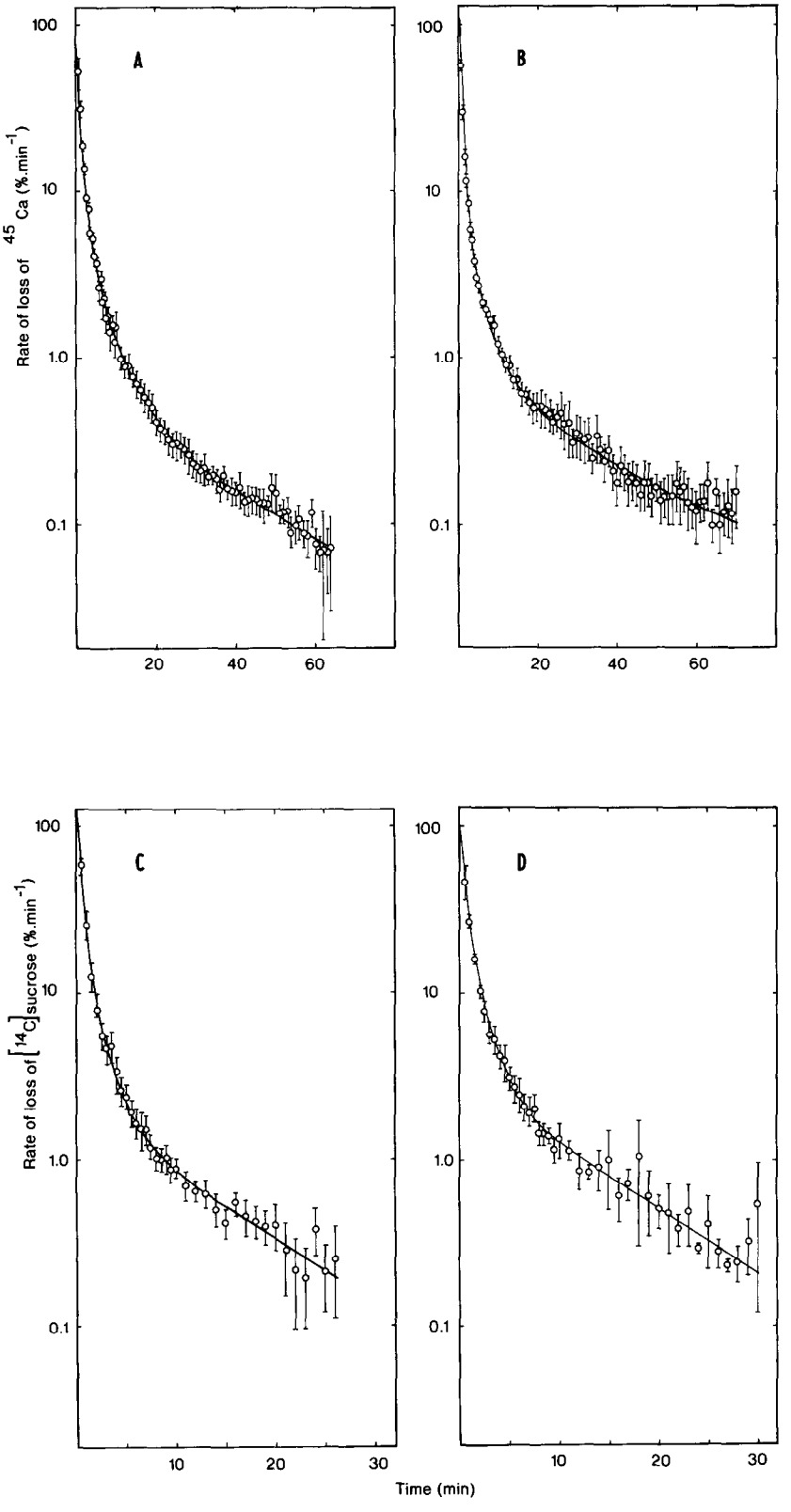


Fig. 2. Semi-logarithmic plots of the rate of loss of ^{45}Ca (A and B) and [^{14}C]sucrose (C and D) from control isolated perfused hearts (A and C) and hearts perfused in the presence of glucagon (2×10^{-7} M). Heart perfusions, ^{45}Ca outflow exchange and measurement of the loss of [^{14}C]sucrose were conducted as described in Materials and Methods. Each data point is the mean ± S.E.M. of 7 (A and B), 6(C) or 4(D) separate experiments. Each solid line represents the values of dq_4/dt calculated from equation (1) using the numerical values of the constants for the line of best fit (Tables 2 and 4).

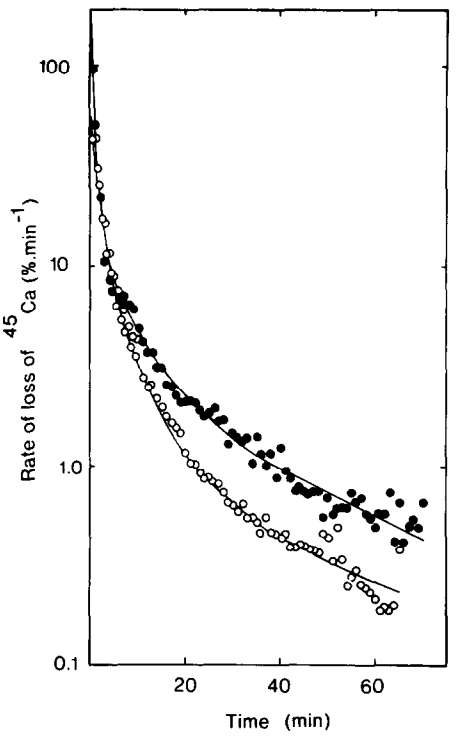


Fig. 3. Semi-logarithmic plots of the rate of loss of ^{45}Ca from the cellular space of isolated hearts perfused in the presence or absence of glucagon. The contribution made by the rate of loss of ^{45}Ca from the extracellular space to the rate of loss of ^{45}Ca from the heart for the data of Fig. 2A for control hearts (○), and Fig. 2B for hearts perfused in the presence of glucagon (●), was subtracted as described in Materials and Methods to give time-courses for the rate of loss of ^{45}Ca from the cellular space. The solid lines represent the values of dq_4/dt calculated from equation (1) using the numerical values of the constants for the line of best fit (Table 2).

outflow. Similar assumptions have been made by others in the interpretation of data from ^{45}Ca exchange experiments in cardiac muscle preparations [18–21].

The simplest system of kinetically-distinct compartments of exchangeable calcium which is consistent with the data for ^{45}Ca outflow exchange from the cellular space in both control and glucagon-treated hearts is one in which three compartments are associated with cardiac muscle cells. Since [^{14}C]sucrose was used to monitor the loss of ^{45}Ca from the extracellular space, ^{45}Ca associated with the cellular space will include calcium bound to extracellular sites on cardiac muscle cells as well as intracellular calcium (i.e. that present in the sucrose-impermeable space). Each of the three kinetically-distinct compartments of exchangeable calcium may be composed of a number of different anatomical compartments which, to a first approximation, are kinetically homogeneous.

The values obtained for the fractional transfer rates (rate constants, k_{0j}) for the outflow of calcium from compartments 1, 2 and 3 in control rat hearts (0.61, 0.14 and 0.02 min^{-1}, respectively) are in reasonable agreement with the values for these parameters of: 0.5, 0.13 and 0.024 min^{-1}, obtained for rabbit interventricular septum [20]; 0.87, 0.16 and 0.014 min^{-1} for dog papillary muscle [19]; and 0.87 and 0.11 min^{-1}, calculated from data obtained for guinea pig atria [21]. On the basis of the results reported by Langer and his co-workers [22–24], it is concluded that compartment 1 (k_{01} = 0.61 min^{-1}) is chiefly composed of calcium bound to the glycocalyx of cardiac muscle cells, although it may also include rapidly exchangeable calcium in the myoplasm. Comparison of the value obtained for the fractional transfer rate for compartment 3 (k_{03} = 0.02 min^{-1}) with the results of experiments in which the quantity of ^{45}Ca present in subcellular fractions of the heart was measured indicates that this com-

Table 5. Distribution of ^{45}Ca between subcellular fractions prepared from control perfused hearts subject to ^{45}Ca outflow exchange*

Fraction	Predominant component†	Amount ^{45}Ca present (% initial dose in heart)		^{45}Ca lost (% initial dose)
		5 min after outflow began	40 min after outflow began	
Total homogenate		19 ± 3	7 ± 3	12 ± 3
1		3.9 ± 1.9	1.0 ± 0.4	2.9 ± 1.9
2	Mitochondria	0.9 ± 0.1	0.4 ± 0.2	0.5 ± 0.2
3	Microsomes (sarcoplasmic reticulum)	0.48 ± 0.06	0.8 ± 0.6	0
4		16.4 ± 2.1	6.2 ± 2.5	10.2 ± 3.3

* Heart perfusions, ^{45}Ca outflow exchange, homogenisation of the hearts and differential centrifugation were conducted as described in Materials and Methods. The subcellular fractions were: 1, the pellet obtained after centrifugation of the homogenate at 4,500 *g* min; 2, the pellet obtained after centrifugation of the supernatant from the previous step at 90,000 *g* min; 3, the pellet obtained after centrifugation of the supernatant from the previous step at 960,000 *g* min; and 4, the supernatant from the previous step. The results are the means ± S.E.M. of three separate experiments.

† Assigned on the basis of the fractionation methods developed by Kerbey *et al.* [17] and Harigaya and Schwartz [15].

partment represents exchangeable calcium present in mitochondria and possibly the sarcoplasmic reticulum. Compartment 2 ($k_{02} = 0.14\ min^{-1}$) may represent exchangeable calcium present in another intracellular store.

The results indicate that glucagon alters the properties of exchangeable calcium present in compartments 1 and 3, but has no discernible effect on exchangeable calcium in compartment 2. The effect on compartment 1 is to increase the fractional transfer rate (k_{01}) for calcium outflow. This observation is consistent with the results of Nayler *et al.* [1] and Meinertz *et al.* [2] who showed, using ^{45}Ca inflow exchange experiments, that glucagon or dibutyryl cyclic AMP increase the amount of ^{45}Ca exchanged at 5–7 min after the exposure of cardiac muscle to ^{45}Ca. However, the present investigation has been able to define the locus of the action of glucagon more clearly.

The main effect of glucagon on compartment 3 is to increase the quantity of exchangeable calcium present in this compartment. Friedmann *et al.* [25] have recently reported that mitochondria isolated from hearts exposed to glucagon exhibit faster rates of calcium uptake than control mitochondria. This observation suggests that the action of glucagon on intact cardiac muscle results in stimulation of the transport of calcium into mitochondria which may, in turn, lead to the increased quantity of exchangeable calcium observed in compartment 3 in the present experiments.

Acknowledgements—This work was supported by grant G1292/1120 from the National Heart Foundation of Australia. We are grateful to Suzanne Lamont for skilled assistance, and to Dr. John C. Wadsworth, C.S.I.R.O. Division of Animal Production, Blacktown, New South Wales, for advice on use of the SAAM computer programme.

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