# Dietary Selenium and Vitamin E Intakes Alter β-Adrenergic Response of L-Type Ca-Current and β-Adrenoceptor-Adenylate Cyclase Coupling in Rat Heart[1]

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ABSTRACT Previously we have shown that both insufficient (combined with vitamin E deficiency) and excess intake of selenium (Se) impairs isoproterenol (ISO)-induced contractions of rat papillary muscle. In the present study, we used patch-clamp and biochemical techniques to investigate mechanisms of this effect in rats fed a Se- and vitamin E-deficient, a Se-excess or a normal diet. Whole-cell configuration of patch-clamp technique was used to investigate L-type $Ca^{2+}$ currents ($I_{Ca,L}$) and their regulation by β-adrenergic receptor stimulation in enzymatically isolated single rat ventricular myocytes. Alteration of Se and vitamin E intake did not affect peak $I_{Ca,L}$, but the threshold potential of activation was significantly different among groups. Maximal $I_{Ca,L}$ responses to ISO were depressed in both experimental groups, but the $EC_{50}$ values were not affected. In the Se-deficient group, basal, ISO- or forskolin-induced adenylate cyclase (AC) activity, measured in cardiac membrane preparations, was reduced when compared to the control, whereas 5′ guanylyimidodphosphate (GppNHp) stimulated activity was unaffected. Decreased β-adrenoceptor density and reduced GppNHp-induced affinity shift in ISO binding were also observed in the deficient group. No such differences were present in the excess group. These results suggest that combined Se and vitamin E deficiency interferes with β-adrenoceptor-AC coupling, whereas excess intake of Se does not affect it. Thus, in the deficient group, the impairment of $I_{Ca}$ responses to ISO may be a result of a defect in β-adrenoceptor-AC pathway. Impairment of $I_{Ca}$ response in the excess group, however, appears to have a different underlying mechanism. J. Nutr. 130: 733–740, 2000.

KEY WORDS: • *trace element* • *selenium* • *vitamin E* • *receptor-adenylate cyclase coupling* • *rat cardiac myoctes*

Selenium (Se)[3] plays an important role in mammalian physiology. Severe dietary Se deficiency produces a set of distinct pathologies, depending on the species and the adequacy of vitamin E. Pathological signs were more pronounced when levels of vitamin E and other antioxidants were also altered (Leibovitz et al. 1990, Sword et al. 1991). In a certain region of China, severe Se deficiency has been associated with an endemic disease known as Keshan disease (Li et al. 1985), which is characterized by congestive heart failure. There are also several epidemiological studies in Europe linking low serum Se levels to cardiac disease (Salonen et al. 1985 and 1988). Se supplementation has been suggested (Neve 1991) and indeed shown to have a protective effect during myocardial ischemia (Poltronieri et al. 1992). Se deficiency also causes a number of different symptoms in animal models (Levander 1986).

Chronic Se toxicity affects the major organs including liver, spleen, kidney and heart in experimental animals; especially sodium selenite has been shown to cause cellular dysfunction in a number of cells and tissues including erythrocytes (Young et al. 1981), hepatocytes (Anundi et al. 1982), lens (Hightower and McCready 1991, Wang et al. 1993Lin-Shiau et al. 1989) and heart muscle (Turan et al. 1996). Selenium toxicity in humans is rare, yet it occurs with symptoms similar to those in animals (Yang et al. 1983).

The mechanism of Se toxicity, as well as its deficiency, is not clearly understood. The toxicity of Se likely is related to its ability to form covalent linkages with intracellular proteins (Dickson and Tappel 1969). Selenite catalyzes oxidation of glutathione (Tsen and Tappel 1958) and can react intracellularly with other sulfhydryl compounds (Björnstedt et al. 1992, Frenkel et al. 1991, Handel et al. 1995, Yang and Yang 1989). On the other hand, most pathologies associated with Se deficiency appear to be directly attributable to increased free-

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[3] Abbreviations used: AC, adenylate cyclase; GppNHp, 5′ guanylyimidodphosphate; PKA, protein kinase A; ISO, isoproterenol; $I_{caL}$, L type calcium current; [$^{125}$I]-IPIN, [$^{125}$I]-iodopindolol.

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radical damage in tissues (Bettger 1993, Burk 1989 and 1990, Hoekstra 1975, Levander 1986, Sunde 1990).

In earlier studies, it was pointed out that Se and vitamin E are nutrients which are interrelated in their metabolic functions (Schwarz 1965, Tappel 1965). Se deficiency alone did not affect the electrophysiological or mechanical functions of rat hearts (Ringstad et al. 1988, Ytreus et al. 1988), whereas the combined deficiency of Se and vitamin E leads to some abnormalities in cardiovascular functions of both humans and laboratory animals (Chen et al. 1980, Grupp et al. 1983, Oldfield 1987, Olson and Kobayoshi 1992, Shamberger 1983). In a recent study, using electrically stimulated papillary muscles isolated from rats fed with a Se and vitamin E-deficient diet, we demonstrated that isoproterenol (ISO)-induced facilitation of muscle contraction was smaller than that observed in muscles from control animals. A similar decrease in $\beta$-adrenergic response was also observed in rats fed with a Se excess diet (Turan et al. 1999).

In the present study, to further investigate the effects of combined Se and vitamin E deficiency or Se excess on $\beta$-adrenergic responses in cardiac cells, we fed one group of rats with a combined Se and vitamin E-deficient diet (deficient group) and another with a diet containing excess Se (excess group). In these groups, we investigated molecular mechanisms that may underlie the changes in responsiveness of cardiac muscle to adrenergic stimulation. We used whole-cell patch-clamp technique to study the L-type $Ca^{2+}$ current in isolated cardiac myocytes (a slow inward $Ca^{2+}$ current, $I_{Ca,L}$, which is characterized by activation at relatively more depolarized potentials and is the dominant $Ca^{2+}$ current in all mature cardiac myocytes from rats). Also the modulation of this $I_{Ca,L}$ by $\beta$-adrenergic stimulation was studied. We additionally used some biochemical techniques to obtain information about the state of coupling between $\beta$-adrenoceptors and adenylate cyclase, and the receptor density in cardiomyocytes.

## METHODS

***Animals and housing.*** Weanling Wistar rats (Ankara University Medical Faculty, Animal Care Facility) of either sex were divided randomly into three groups and housed in stainless steel, wire-bottomed cages initially at a density of three per cage and as they grew, were then caged individually. They were maintained at an ambient air temperature of 22 ± 1°C and a 12-h light/dark cycle. All procedures used in the experiments were approved by the Ethics Committee of Ankara University Medical School.

***Diets and feeding.*** The deficient diet was obtained commercially. The basal diet was a Se- and vitamin E-deficient torula yeast-based diet. It contained 30% torula yeast, 0.3% DL-methionine, 58.7% sucrose, 2% corn oil, 5% Se-deficient AIN-76 TM mineral mix, 1% vitamin E-deficient vitamin mix AIN-76 TM (Horland Tekland, Madison, WI; Koukay et al. 1990). Se and vitamin E were supplemented in adequate and rich diets with sodium selenite and $\alpha$-tocopherol acetate (Sigma). Selenium content of the diets was determined by using a graphite furnace atomic absorption spectrometer (Varian Atomic Absorption Spectrometer AA-30/40, Sydney, Australia). Based on analysis of random batches of diet, Se concentration of the deficient diet was 9.8 $\mu$g Se/kg, and the adequate and rich diets contained 225 $\mu$g Se/kg-diet and 4.2 mg Se/kg, respectively. Deionized water was given to the rats, which contained very little Se (<1 $\mu$g/L). The rats were fed either an adequate diet (control group), a deficient diet (Se- and vitamin E-deficient group) or an excess diet (Se-excess group). The rats were permitted free access to the food and water for 12–14 wk.

***Tissue preparation and analysis for Se levels.*** Rats were anesthetized with a mixture of sodium pentobarbital (30 mg/kg) and heparin. Blood samples for Se and vitamin E determinations were collected by cardiac puncture. The thorax was opened and hearts were removed for determinations of Se levels. Plasma vitamin E concentrations were determined by HPLC (McMurray and Blanchflower 1979). Se levels were measured using a Zeeman graphite furnace atomic absorption spectrometer (AA-30/40 Varian Spectrophotometer) (Berly and Gillian 1988). As reported previously (Turan et al. 1999), Se concentrations in plasma, erythrocytes and heart homogenates (as mean ± SD) were 4.21 ± 1.16 $\mu$mol/L, 11.06 ± 1.08 nmol/g hemoglobin and 4.83 ± 0.15 nmol/g wet weight, respectively, in the control group; 2.16 ± 1.13 $\mu$mol/L, 4.11 ± 0.34 nmol/g hemoglobin and 1.90 ± 0.11 nmol/g wet weight, respectively, in the deficient group; 11.91 ± 1.08 $\mu$mol/L, 17.5 ± 1.31 nmol/g hemoglobin, and 16.03 ± 0.60 nmol/g wet weight, respectively, in the excess group. Plasma vitamin E levels in deficient rats were 50% of the control animals.

***Isolation of ventricular myocytes.*** Myocytes were isolated according to the method of Wittenberg et al. (1986). Briefly, hearts were perfused at 37°C with a HEPES-buffered solution containing (in mmol/L): NaCl 123, KCl 5.4, $NaHCO_3$ 5, $NaH_2PO_4$ 2, $MgCl_2$ 1.6, glucose 10, taurine 20, HEPES 20, pH 7.1 and bubbled with 100% $O_2$. After 5 min, hearts were perfused with the same buffer containing 0.8–1.5 g/L collagenase with a perfusion rate of 8–10 mL/min for 10–30 min. At the end of the collagenase perfusion, a piece of left ventricle was cut off and stirred to obtain cells. The cells were then suspended at 37°C in the HEPES-buffered solution containing 1 mmol/L $CaCl_2$ and 0.5% bovine serum albumin. The myocytes were kept under this condition and used in experiments within 4–6 h. Generally 5–7 × $10^6$ cells were obtained from control rats. Usually 70–80% of these cells were well relaxed as assessed by microscopic inspection. Cell yields from both of the altered diet groups were much lower than the control group.

***Recording of $Ca^{2+}$ currents from whole-cell patch clamp.*** To record $Ca^{2+}$ current ($I_{Ca,L}$), the ventricular myocytes were placed in a tissue culture dish and superfused by gravity with a solution containing (in mmol/L): CsCl 20, NaCl 117, $CaCl_2$ 1.8, $MgCl_2$ 1.7, glucose 10 and HEPES 10, pH 7.4. The current recordings were performed under the presence of tetrodotoxin (50 $\mu$mol/L) and cesium in the superfusing solution to inhibit the $Na^+$ and $K^+$ currents, respectively. The internal solution in the patch electrode (resistance of 1.5–3.5 MΩ) contained (in mmol/L): CsCl 120, $MgCl_2$ 6.8, $Na_2ATP$ 5, $Na_2$-creatine phosphate 5, $Na_2GTP$ 0.4, EGTA 5, $CaCl_2$ 0.06 and HEPES 20, pH adjusted to 7.2 with CsOH. Once a cell was sealed to the electrode, it was lifted up and exposed to different extracellular solutions, containing different concentrations of ISO (in the range of 0.1 nmol/L to 1 $\mu$mol/L), by positioning the pipette at the extremity of one of the six capillaries flowing at a steady rate. Experiments were carried out at room temperature (22 ± 1°C).

Whole cell currents ($I_{Ca}$) were recorded using a RK-300 (Biologic, Grenoble, France) patch-clamp amplifier. For the recording and analysis of the currents, we used a homemade data acquisition program written in Pascal, called PCSoft. To monitor $I_{Ca}$, ventricular myocytes were depolarized from −70 mV holding potential to 0 mV for 200 ms every 4 s. Current recordings were filtered at 1 kHz and sampled at a rate of 10 kHz (12-bit A/D converter, Advantech PCL-818PG; Advantech Co., Taipen, Taiwan). $I_{Ca}$ amplitude was measured as the difference between peak inward current and the current at the end of 200 ms voltage pulse. Rundown of peak $I_{Ca}$ was limited, generally to 10% within 30 min. The fast and slow inactivation constants of $I_{Ca}$ were calculated by fitting the data to a sum of two exponential functions by a homemade program using a simplex algorithm. This homemade data acquisition system monitors continuously the series resistance ($R_s$), the membrane resistance ($R_m$) and the membrane capacitance ($C_m$). This system has also an advantage to stop current recording to avoid acquisition of data when the $R_s$ > 10 MΩ, which can be misleading due to the high series resistance. In our study, the $R_s$ ranged from 4 to 9 MΩ. The series resistance and the membrane capacitance were not compensated during current recordings.

***Membrane preparation.*** Crude myocardial membranes were prepared from control, Se-deficient and Se-excess groups ($n$ = 7, 7 and

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9, respectively) by homogenizing whole hearts (using Ultraturax with 3 × 20 s bursts at maximum speed) in ice-cold lysis buffer (10 mmol/L Tris-HCl, pH 7.4, 1mmol/L EDTA, 0.2 mmol/L phenylmethylsulphonylfloride, aprotinin 5 mg/L). The homogenate was centrifuged at 500 × *g* for 15 min, and the supernatant was used to obtain membrane pellet following a centrifugation at 45,000 × *g* for 30 min. Membranes were washed twice with buffer (50 mmol/L Tris-HCl pH: 7.4, 10 mmol/L $MgCl_2$, 1 mmol/L EDTA, 2 mmol/L dithiothreitol, 0.2 mmol/L phenylmethylsulphonylfloride, aprotinin 5 mg/L) and stored (3 g/L of protein) in the same buffer including 25% sucrose at −45°C until assayed for receptor binding, adenylate cyclase activity or Western blotting.

***Binding experiments.*** Competition binding of [$^{125}$I]-iodopindolol ([$^{125}$I]-IPIN, 80 pmol/L) (synthesized by using chloaramine T method and purified on reverse-phase HPLC to a specific activity of 7400 × $10^{10}$ Bq/mmol) (Barovsky and Brooker 1980, Wolf and Harden 1981) with isoproterenol in the presence or absence of 5′guanylyimidodiphosphate (GppNHp) was measured in triplicate after equilibrating the binding reaction (40 μg membrane protein) in binding buffer for 60 min at 37°C (50 mmol/L TRIS at pH 7.4 and 100 mmol/L KCl) in a total volume of 100 μL. Reactions were terminated by adding 3 mL of ice-cold binding buffer and rapid filtration through Whatman GF/B filters using a vacuum filtration manifold (Millipore, Bedford, MA). Filters were washed twice with 4 mL of ice-cold binding buffer and counted for radioactivity.

In order to measure β-adrenoceptor density in the heart membranes, single concentration of [$^{125}$I] IPIN was used at a concentration of 800 pmol/L (80 times of its Kd value) to saturate β-adrenoceptors. Nonspecific binding was determined in the presence of 100 μmol/L of unlabeled propranolol and specific binding was calculated as the difference between total and nonspecific bindings.

***Adenylate cyclase assay.*** Adenylate cyclase assay was performed using 5 μg membrane protein in a total volume of 100 μL in triplicate or quadruplicate as described previously (Ugur and Onaran 1997). Briefly the assay was started by adding cyclase buffer (final: 50 mmol/L Tris, pH 7.4, 100 mmol/L KCl, 1.6 mmol/L $MgCl_2$, 0.5 mmol/L ATP, 1 mmol/L isobutylmethylxanthine, 5 mmol/L phosphoenolpyruvate and 5 x $10^3$ U/mL pyruvate kinase) onto the membranes that were equilibrated for 5 min with various stimuli at 37°C. The reaction was stopped after 10 min by adding 100 μL of 0.2 mol/L HCl. Accumulated cyclic AMP (cAMP) was measured by radioimmunoassay using acetylation protocol. Accumulation of cAMP per mg membrane protein per minute was considered as adenylate cyclase activity.

***Immunoblotting.*** The expression level of Gsα protein in the membranes was assessed by Western blotting. SDS-PAGE (%10) was performed according to the method of Laemmli (1970). We used anti-Gsα primary antibody (RM/1; New England Nuclear, Boston, MA) to label Gsα on the blotting membrane and visualized the antibody-bound Gsα by using ECL (Amersham). Protein concentration in the membrane preparations was determined according to the Bradford method (Bradford 1976).

***Statistical analysis.*** All data are reported as mean ± SEM. Student's *t* test was used for the comparison of means. Probability values $< 0.05$ were considered significant.

## RESULTS

***Characteristics of $I_{Ca,L}$ in hearts of rats fed control, se- and Vitamin E-deficient and Se-excess diets.*** Voltage dependence of the L-type calcium current ($I_{Ca,L}$) recorded from isolated cardiac cells were different in Se-excess and Se-deficient diet groups when compared to the control group. For both experimental groups, threshold potential for activation was significantly lower than the control group ($P < 0.05$), and potential for maximum activation was higher than the control group ($P < 0.05$) but the maximum current value was not different (**Fig. 1**, **Table 1**).

***Effect of β-adrenergic stimulation on $I_{Ca,L}$.*** At most, three cumulative doses of ISO were used for lower concentrations (from 0.1 to 10 nmol/L). Noncumulative doses were used for higher concentrations to avoid desensitization. In control cells, 1 μmol/L ISO caused a 90 ± 3.3% maximal increase in $I_{Ca}$ over its baseline value (**Fig. 2**). Meanwhile deficient and excess groups showed a maximal increase of only 20 ± 3.4 and 30 ± 3.6%, respectively. These two values were significantly less than the control values ($P < 0.001$). However, the $EC_{50}$ values, calculated from dose-response curves shown in Figure 2, were not different among groups (30, 34 and 32 nmol/L in control, deficient and excess groups' cells, respectively).

***Kinetics of $I_{Ca}$.*** In heart cells from both experimental groups, time to peak ($T_p$) and two inactivation time constants ($\tau_s$ "slow" and $\tau_f$ "fast") for $I_{Ca}$ (estimated from the current

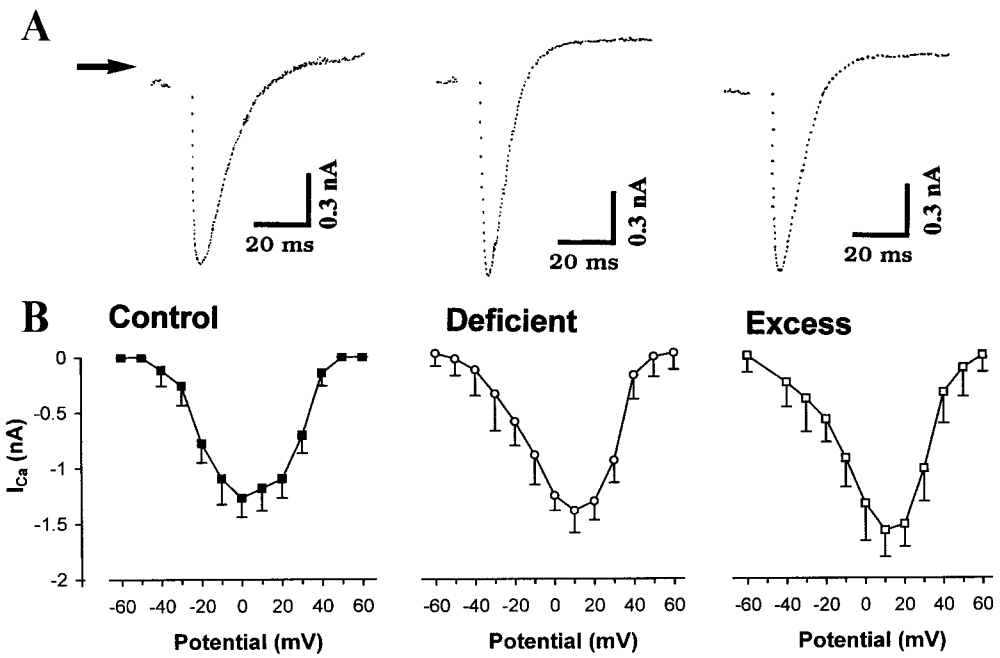



**FIGURE 1** Calcium currents recorded from cardiac myoctes of rats fed with a control, deficient or excess diet. *A:* Representative calcium current ($I_{Ca}$) traces obtained with depolarization from −70 to 0 mV for; 200 ms, in control (membrane capacitance ($C_m$) for this cell is 178 pF), deficient ($C_m$ is 156 pF) and excess group heart cells, ($C_m$ is 186 pF), as indicated. The arrow represents the zero current. *B:* Graphs showing average current-voltage relations for peak $I_{Ca}$ (measured as the difference between the peak $I_{Ca}$ current and at the end of 200 ms depolarization) elicited from a holding potential of −70 mV in control, deficient and excess group cells. Values are shown as average and SEM, the number of cells used are 9, 11 and 10 for control, deficient and excess groups, respectively, at least seven different animals were used from each group.

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**TABLE 1**

*Characteristics of calcium current in isolated rat heart ventricular cells*[1]

| Group | *n* | Peak $I_{ca,L}$ (pA) | Threshold potential for activation (mV) | Potential for maximal activation (mV) |
|---|---|---|---|---|
| Control | 9 | 1267 ± 165 | −39.5 ± 2.2 | +0.4 ± 1.3 |
| Se- and vit. E-deficient group | 11 | 1424 ± 169.1 | −58.5 ± 1.8* | +12 ± 1.8* |
| Se-excess group | 10 | 1594 ± 220.1 | −61.2 ± 2.2* | +10.8 ± 1.9* |

[1] Maximum amplitude of L-type calcium current (Peak $I_{ca,L}$) elicited by depolarizing the cell to 0 mV or +10 mV from a holding potential of −70 mV. Threshold potential for actvation of $I_{ca,L}$ and the value of depolarization potential that maximum current can be elicited (potential for maximal activation). Values are means ± SEM. Cells were from control, selenium and vitamin E-deficient and selenium-excess group rats. *n*, number of cells from each group. * $P < 0.05$ compared with the control group. Abbreviations: Selenium (Se), L type calcium current ($I_{Ca,L}$).

induced by the voltage pulse from –70 to 0 mV for 200 ms) were all significantly less than the control group values ($P < 0.01$), but were not different from each other (**Table 2**, see also Fig. 2 upper panel). In any group, 1 μmol/L ISO did not alter $T_p$, $\tau_s$ and $\tau_f$ values significantly (Table 2).

***Adenylate cyclase assays.*** Basal cyclase activity and cyclase activity induced by several stimuli are shown in **Figure 3**. To eliminate interday variations from the data and make the results easier to interpret, cyclase activity is given, for each stimulus, as the difference between experimental and control

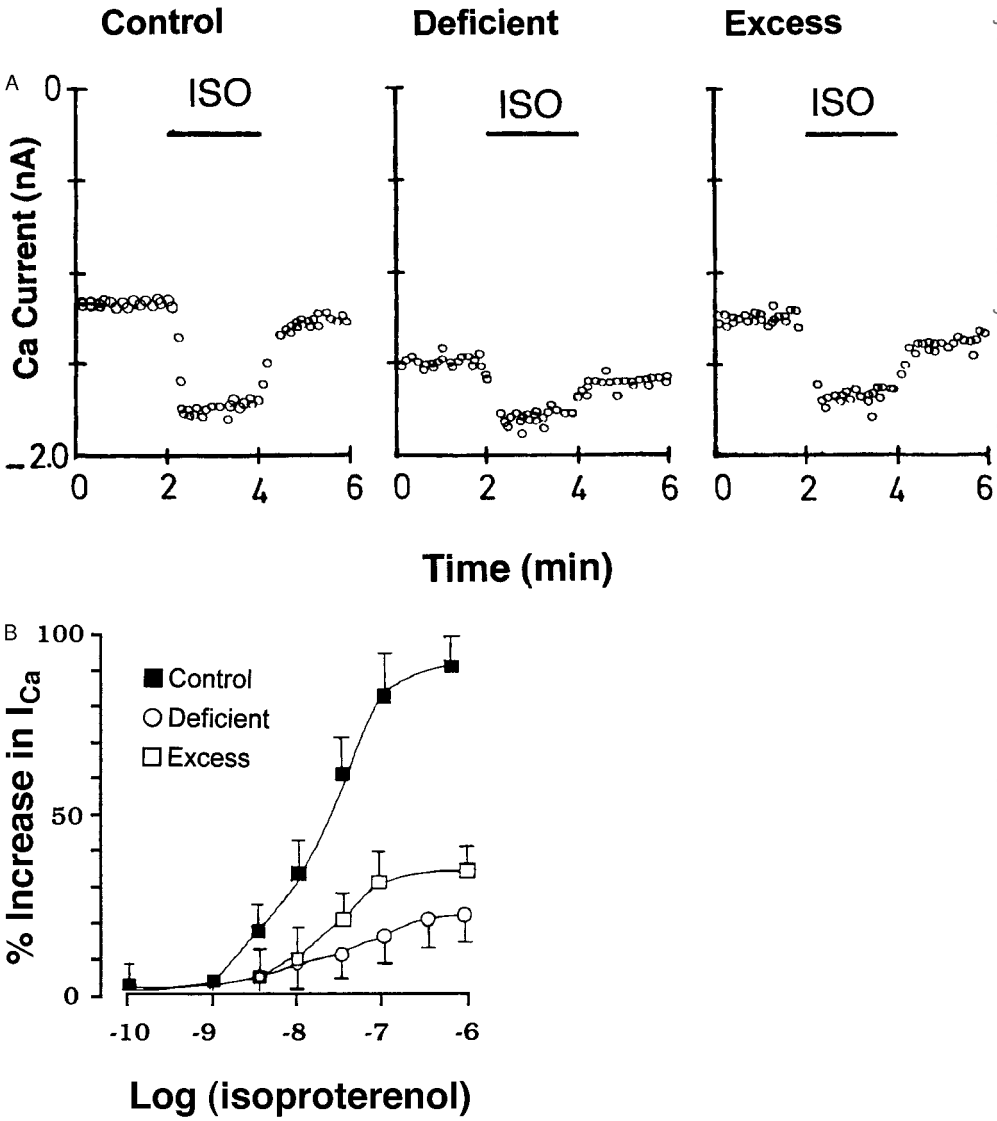



**FIGURE 2** Effect isoproterenol (ISO) on calcium (Ca) currents recorded from cardiac myoctes of rats fed with control, deficient or excess diet. *A:* Representative traces showing the time course of the effect of ISO on peak calcium current ($I_{Ca}$) amplitude in control, deficient and excess group cells as indicated. The depolarization pulse which $I_{Ca}$ is elicited with and the calculation of peak $I_{Ca}$ are as described for the previous figure. Solid line represents the duration of ISO application. Cell membrane capacitance ($C_m$) for cells from control, deficient and excess groups are 155 pF, 210 pF and 210 pF, respectively. *B:* Dose response curves for the effect of ISO on peak $I_{Ca}$ in control, deficient and excess group cells. The ISO-induced increase in $I_{Ca}$ is plotted as a percentage of the basal $I_{Ca}$. Values are shown as means and SEM (error bars). Number of rats used for cell isolation for each group are six for control, seven for deficient and five for excess, groups, and at least seven different cells were used for each ISO concentration.

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**TABLE 2**

*Affect of 1 μmol/L isoproterenol on calcium current kinetics at 0 mV*[1]

| | Control | Control + ISO | Se and vit. E-deficient | Se and vit. E-deficient + ISO | Se-excess | Se-excess + ISO |
|---|---|---|---|---|---|---|
| *n* | 9 | 7 | 11 | 7 | 10 | 7 |
| $T_p$, *ms* | 9.8 ± 0.2 | 10.5 ± 0.2 | 11.0 ± 0.1 | 10.8 ± 0.2 | 10.0 ± 0.1 | 10.2 ± 0.1 |
| $\tau_f$, *ms* | 9.3 ± 0.3 | 10.9 ± 0.3 | 6.5 ± 0.5* | 7.2 ± 0.3* | 6.5 ± 0.4* | 6.8 ± 0.2* |
| $\tau_s$, *ms* | 58.1 ± 1.9 | 59.1 ± 1.2 | 37.9 ± 1.2* | 25.8 ± 0.9* | 37.9 ± 0.9* | 28.8 ± 1.8* |

[1] Time to reach peak current ($T_p$), slow ($\tau_s$) and fast ($\tau_f$) inactivation constants of $I_{Ca,L}$, with and without ISO application. Values are means ± SEM. At least seven ventricular myocytes from different rats in each group were used. Cells were from control, selenium and vitamin E-deficient, and selenium-excess group rat hearts. $I_{Ca,L}$ inactivation was described by the equation $I_{Ca,L} = A_f e^{-(t/\tau_f)} + A_s e^{-(t/\tau_s)} + A_{ss}$, where $\tau_f$ and $\tau_s$ are the time constants of the fast and slow components respectively, and t is time. * $P < 0.01$ compared with the control group. Abbreviations: L type calcium current ($I_{Ca,L}$), isoproterenol (ISO).

groups, normalized to the actual activity of the control (values from the control group are measured on the same day and in parallel to the other two groups).

Basal AC activity of the membranes from deficient group was lower than the control group ($P < 0.01$), but the activity of the membranes from excess group was not different from the control. Likewise, the ISO- or forskolin-induced increase in AC activity was less ($P < 0.01$) in deficient group membranes but did not differ in membranes of the control and excess groups. Induction of AC activity by GppNHp did not differ in all three groups.

***Binding experiments.*** Saturation binding experiments were performed in these membranes to determine the total β-adrenoceptor number. We also investigated the GppNHp-induced shift in agonist affinity (in agonist competition experiments) as a measure of the efficiency of receptor-G-protein coupling.

Saturation binding experiments showed no significant difference in the number of [$^{125}$I] IPIN binding sites between control and excess group membranes (36.36 ± 3.15 and 40.75 ± 3.84 fmol/mg protein, respectively), but binding sites were significantly lower in the deficient group (27.29 ± 3.01 fmol/mg protein) compared to the control (%73 of control membranes) ($P < 0.05$).

In agonist competition experiments, in all groups, agonist affinity measured in the presence of GppNHp (10 μmol/L) did not differ among control, deficient and excess groups. However, GppNHp induced a significant shift in agonist $IC_{50}$ in the control and Se excess groups, but not in the Se-deficient group (calculated using ALFIT by using extra sum of squares principle; De Lean et al. 1978) (see **Fig. 4**). Shifts in log $IC_{50}$ values were as follows: 1.000 ± 0.224 ($P < 0.001$), 0.400 ± 0.245 ($P = 0.12$) and 0.600 ± 0.212 ($P = 0.01$) in control, deficient and excess groups, respectively.

***Gs content of the membranes.*** We performed Western blot analysis with cardiac membranes using RM-1, an antibody specific to α subunit of Gs, to compare the Gs content of the membranes from all three groups. Gs antibody identified two membrane protein bands in all membranes; one with the apparent molecular mass of 45 KD and the other 52 KD. Both of these bands appeared to be the same in all three groups (**Fig. 5**).

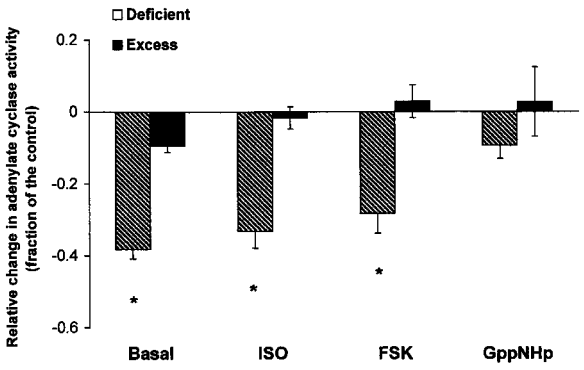



**FIGURE 3** Adenylate cyclase activity measured in cell membranes from indicated groups in the presence of indicated stimuli: Isoproterenol (ISO)$10^{-6}$ mol/L, forskolin (FSK) $10^{-6}$ mol/L, 5′-guanylyimidodiphosphate (GppNHp) $10^{-5}$ mol/L. Data are represented in the following way: for each stimulus, in parallel experiments, differences between cyclase activities obtained in control and indicated group (deficient or excess) are normalized with respect to the value of individual determinations. Mean values of five such determinations for each stimulus (as indicated) are shown. This representation was chosen to eliminate the interday variation in adenylate cyclase determination. Data are represented as means and SEM (five independent triplicate experiments. Membranes were prepared from at least seven different rats from each group). * Indicates significant difference from zero ($P < 0.01$).

## DISCUSSION

In the present study, we have shown that the amplitudes of L-type $Ca^{2+}$ currents ($I_{Ca,L}$) recorded in isolated cardiac myocytes from rats fed with a Se- and vitamin E-deficient or Se-excess diet were not significantly different from those measured in the control group. However, kinetics and I-V relations of $I_{Ca,L}$ were different in the experimental groups compared to the control. Maximum $I_{Ca,L}$ responses to ISO, when compared to control cells, were less for deficient and excess groups with no change in $EC_{50}$ values. Total cardiac β-adrenoceptor number and basal or stimulated (with either ISO or forskolin) AC activities were less only for the deficient group (i.e., these variables for the excess group were not different from the control values). In addition, the GppNHp-induced shift in apparent agonist affinity was insignificant only in the deficient group. Gsα content of the membranes, on the other hand, was similar in all groups.

We have previously shown that in rats, altering the Se content (along with vitamin E) of the diet causes a diminished β-adrenergic response in electrically stimulated papillary mus-

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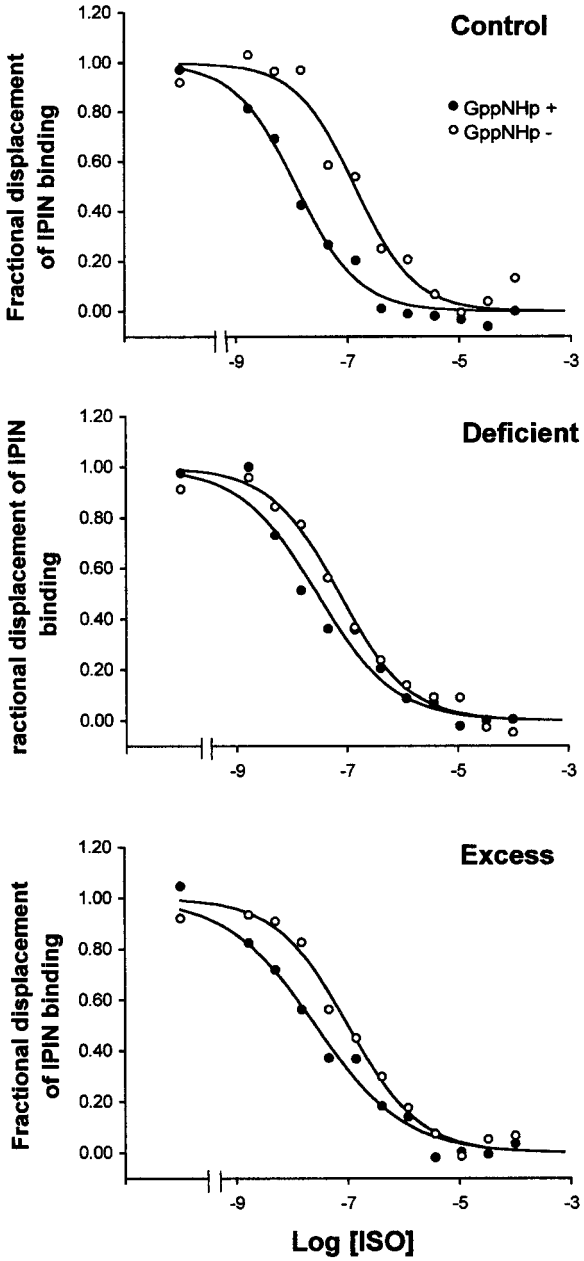



**FIGURE 4** (-)-Isoproterenol-induced displacement of $^{125}$I-iodopindolol binding in the presence or absence of 10 $\mu$mol/L 5′ guanylylimidodphosphate (GppNHp), in heart membranes from control, Se and vitamin selenium (Se)-deficient and Se-excess groups. Solid curves represent best fit of four-parameter logistic equation obtained using ALLFIT. Parameters that show maximum and minimum bindings were shared for the data obtained with or without GppNHp during the fitting procedure. Parameter sharing did not significantly change the goodness of fit ($P > 0.05$) as assessed by ALLFIT, which means that the presence of GppNHp did not affect maximum or nonspecific binding (i.e., the minimum binding value in the saturating presence of the competitor). Therefore, we showed the binding scale normalized with respect to the minimum and maximum bindings of individual curves. Each data point represents mean value of triplicate measurements. The same membrane preparations as in Figure 3 were used.

cle preparations. The muscles from rats fed a Se- and vitamin E-deficient or a Se-excess diet showed a significantly lower maximum response to ISO. The electrically driven contractions in the absence of ISO (i.e., basal contractions), however, were observed to be the same for control and experimental groups (Turan et al. 1999). This observation may suggest that Se deficiency or excess does not interfere directly with the contractile machinery itself, but instead affects the signal transduction pathway of the $\beta$-adrenoceptors (including adrenoceptors, G-proteins, adenylate cyclase (AC), protein kinase A (PKA) and Ca-channel) present in the cardiac tissue and thus depress the ISO response.

It should be noted that although T-type $Ca^{2+}$ channels are absent in adult rat cardiac myocytes and the TTX concentration used in this study blocks $Na^+$ currents effectively, the apparent change in the $I_{Ca,L}$ threshold potential for activation may be due to emergence of T-type $Ca^{2+}$ channels or to alteration of $Na^+$ channel activity induced by the changes in the diet. This point requires further study to clarify.

The present results indicate an alteration in the signaling pathway of $\beta$-adrenoceptors in the deficient rats. First of all, the total $\beta$-adrenoceptor number on the cardiac membranes of these animals appeared to be about 30% lower than the control rats. Secondly, ISO inducible AC activity was decreased, and finally the GppNHp-induced shift in apparent agonist affinity disappeared. Although in the deficient group, GppNHp-induced AC activity was similar to that obtained in the control group, forskolin-induced activity was less. A straightforward interpretation of the latter result can be a decrease in the number of AC molecules in the membrane. However, this interpretation is inconsistent with the fact that GppNHp can induce full cyclase activity in the deficient group. Another explanation can then be a reduced coupling between Gs and $\beta$-adrenoceptor. This interpretation is consistent with the decrease in GppNHp-induced shift in apparent agonist affinity seen in these membranes. Such an interpretation is actually consistent with the whole picture obtained in the Se-deficient group: i) As has been shown in the experiments with negative antagonists, the spontaneous coupling between receptor and Gs contributes, at a certain extent, to basal cyclase activity (Lefkowitz et al. 1993), which is also the case for the present membrane preparations (data not shown). Thus, a reduction in receptor-Gs coupling is expected to result in a decrease in the basal cyclase activity, ii) A reduced basal coupling between receptor and Gs also explains the reduction in forskolin-induced cyclase activity observed in deficient group membranes, when we consider the allosteric nature of the interaction between $\beta$-adrenoceptor, Gs, AC and forskolin. It has been suggested that the interaction of Gs with cyclase is required for forskolin to work on AC (Seamon and Daly 1986). Hence, reduction in spontaneous Gs activation by $\beta$-adrenoceptor may reduce the spontaneous coupling between cyclase and Gs, and thus

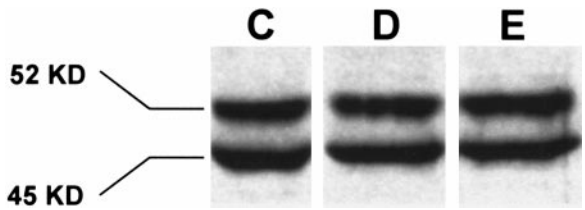



**FIGURE 5** Equal amounts of membrane protein from rats fed control (C), Se- and vitamin E-deficient (D), and Se-excess (E) diets were resolved by SDS-PAGE, and Gs$\alpha$ was detected by immunoblotting with RM-1 antibody. The same membrane preparations as in Figure 3 were used.

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decrease forskolin-induced cyclase activity, iii) Reduced coupling of β-adrenoceptor and Gs may obviously lead to an impairment of isoproterenol-induced cyclase activity and finally, iv) GppNHp-induced activation of cyclase should be insensitive to the reduction of receptor-Gs coupling, which was experimentally the case for the deficient group. All the four points fit well with the present observations in the deficient group.

In conclusion, in the heart, the effect of Se and vitamin E deficiency on β-adrenoceptor and AC system is two-fold: i) there is a decrease in β-adrenoceptor number, which, however does not seem to explain all results obtained in deficient group and ii) Coupling between β-adrenoceptor and Gs is decreased. The reduced coupling does not appear to be due to a lowered Gs number, because Western blots of the cardiac membranes showed no appreciable change in the amount of Gs.

On the other hand, in the excess group we did not find any differences in the β-adrenoceptor-AC system. All the variables we investigated appeared to be similar to those in the control rats. For an explanation of the effect of Se excess on the contractile response to ISO, we should consider a mechanism which is distal to the AC.

In cardiac cells, β-adrenergic agonists increase cAMP production through stimulation of AC via Gs. Cyclic-AMP in turn activates PKA which modulates Ca-channels in these cells. Calcium channels and PKA can be the points, distal to the AC, where Se excess may have an effect and decrease the ISO responses in the hearts of the excess group. Interestingly, the effect of ISO on the calcium current was also smaller in both deficient and excess group cells. Although this reduction in the deficient group cell response can be explained by a diminished transduction in β-adrenoceptor and AC system, such an explanation does not appear to be likely in excess group cells. To understand the reduction in excess group cells, two possible explanations can be put forth: i) a change in the Ca-channel itself, induced by Se excess, which renders it resistant to modulation by PKA or ii) a pathology in PKA itself which reduces its activity. Observed change in the kinetics of the $I_{Ca,L}$ may indicate such an alteration in the channel protein or PKA. It should be emphasized that the possibility of a similar alteration in the Ca-channel and/or PKA function cannot be excluded for deficient group cells and requires further investigation.

Our findings indicate a change in basal and ISO stimulated activity of cardiac $I_{Ca,L}$ in rats fed either Se-excess or Se- and vitamin E-deficient diet. In addition to these changes in cardiac currents, β-adrenergic signal transduction machinery seems to be compromised in rats fed the deficient diet. These findings can be helpful not only in understanding cardiac pathologies related to Se- and vitamin E-deficiency and Se toxicity, but they can also help to clarify the role of Se in the physiology of the heart and the processes where it might be involved.

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## LITERATURE CITED

Anundi, I., Hogberg, J. & Stahl, A. (1982) Involvement of glutathione reductase in selenite metabolism and toxicity, studied in isolated rat hepatocytes. Arch. Toxicol. 50: 113–123.

Barovsky, K. & Brooker, G. (1980) (-)-[125I]-iodopindolol, a new highly selective radioiodinated beta-adrenergic receptor antagonist: measurement of beta-receptors on intact rat astrocytoma cells. J. Cyclic Nucleotide Res. 6: 297–307.

Berly, E. J. & Gillian, L. (1988) Direct determination of selenium of graphite furnace atomic absorption spectrometry with deuterium background correction and a reduced palladium modifier: age specific reference changes. Clin. Chem. 34: 709–714.

Bettger, W. J. (1993) Zinc and selenium, site-specific versus general antioxidation. Can. J. Physiol. Pharmacol. 71: 721–724.

Björnstedt, M., Kumar, S. & Holmgren, A. (1992) Selenodiglutathione is a highly efficient oxidant of reduced thioredoxin and substrate for mammalian thioredoxin reductase. J. Biol. Chem. 267: 8030–8034.

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Annal. Biochem. 72: 248–254.

Burk, R. F. (1989) Recent developments in trace element metabolism and function: newer roles of selenium in nutrition. J. Nutr. 119: 1051–1054.

Burk, R. F. (1990) Protection against free radical injury by selenoenzymes. Pharmacol. Ther. 45: 383–385.

Chen, X., Yang, G., Chan, J., Chen, X., Wen, Z. & Ge, K. (1980) Studies on the relationships of selenium and Keshan disease. Biol. Trace Element Res. 2: 91–107.

De Lean, A., Munson, P. J. & Rodbard, D. (1978) Simultaneous analysis of families of sigmoidal curves: Application to bioassay, radioligand, and physiological dose-response curves. Am. J. Physiol. 253: E97–E102.

Dickson, R. C. & Tappel, A. L. (1969) Reduction of selenocystine by cysteine or glutathione. Arch. Biochem. Biophysic. 130: 547–550.

Frenkel, G. D., Falvey, D. & MacVicar, C. (1991) Products of the reaction of selenite with intracellular sulfhydryl compounds. Biol. Trace Elem. Res. 30: 9–18.

Grupp, I. L., Jamall, I. S., Millard, R. W. & Grupp, G. (1983) Effects of dietary selenium deficiency and selenium and calcium supplementation on myocardial contractile force and oubain sensitivity of the rat heart. IRCS Med. Sci. 11: 22–23.

Handel, M. L., Watts, C .K. W., DeFazio, A. & Day R. O. (1995) Inhibition of AP-1 binding and transcription by gold and selenium involving conserved cysteine residues in Jun and Fos. Proc. Natl. Acad. Sci. USA 92: 4497–4501.

Hightower, K. R. & McCready, J. P. (1991) Effects of selenite on epithelium of cultured rabbit lens. Invest. Ophthalmol. Vis. Sci. 32: 406–409.

Hoekstra, W. G. (1975) Biochemical function of selenium and its relation to vitamin E. Federation Proc. 34: 2083–2089.

Koukay, N., Mouhineddine, S., Richard, M. J., Arnaud, J., DeLeiris, J. & Favier, A. (1990) Influence of selenium on lipid peroxidation and cardiac functions in chronically adriamycin-treated rats. In: Antioxidants in Therapy and Preventive Medicine. (Emerit, I., Packer, L., Auclair, C., eds.), Advances in Experimental Medicine and Biology (264), pp. 353–359. Plenum Press, New York.

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.) 229: 680–685.

Lefkowitz, R. J., Cotecchia, S., Samama, P. & Costa, T. (1993) Constitutive activity of receptors coupled to guanine nucleotide regulatory proteins. Trends Pharmacol. Sci. 14: 303–307.

Leibovitz, B., Hu, M. L. & Tappel, A. L. (1990) Dietary supplements of vitamin E, β-carotene-coenzyme Q10, and selenium protect tissues against lipid peroxidation in rat tissue slices. J. Nutr. 120: 97–104.

Levander, O. A. (1986) Selenium. In: Trace Elements in Human and Animal Nutrition (Mertz, W., ed.), pp. 209–279. Academic Press, New York, NY.

Li, G. S., Wang, F., Kang, D. R. & Li, C. (1985) Keshan disease: an endemic cardiomyopathy in China. Human Pathol. 16: 602–609.

Lin-Shiau, S. Y., Liu, S. H. & Fu, W. M. (1989) Studies on the contracture of the mouse diaphragm induced by sodium selenite. Eur. J. Pharmacol. 167: 137–146.

McMurray, C. H. & Blanchflower, W. J. (1979) Application of a high performance liquid chromatographic fluorescence method for the rapid determination of α-tocopherol in the plasma of cattle and pigs and its comparison with direct fluorescence and high performance liquid chromatography-ultraviolet detection methods. J. Chromatogr. 178: 525–531.

Neve, J. (1991) Physiological and nutritional importance of selenium. Experientia. 47: 187–193.

Oldfield, J. E. (1987) The two faces of selenium. J. Nutr. 117: 2002–2008.

Olson, J. A. & Kobayoshi, S. (1992) Antioxidants in health and disease: overview. Proc. Soc. Exp. Biol. Med. 200: 245–247.

Poltronieri, R., Cevese, A. & Sbartani, A. (1992) Protective effect of selenium in cardiac ischemia and reperfusion. Cardioscience. 3: 155–160.

Ringstad, J., Tande, P. M., Norheim, G. & Refsum, H. (1988) Selenium deficiency and cardiac electrophysiological and mechanical function in the rat. Pharmacol. Toxicol. 63: 189–192.

Salonen, J. T., Salonen, R., Pentila, I., Herranen, J., Tauhiainen, M., Kantola, S., Lappetelainen, R., Maenpaa, P., Alfthan, G. & Puska, P. (1985) Serum fatty acids, apolipoproteins, selenium and vitamin antioxidants and the risk of death from coronary artery disease. Am. J. Cardiol. 56: 226–231.

Salonen, J. T., Salonen, R., Seppanen, K., Kantola, S., Parviainen, M., Alfthan, G. & Maenpaa, P. (1988) Relationship of serum selenium and antioxidants to

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plasma lipoproteins, platelet aggreability and prevalent ischaemic heart disease in Eastrean Finnish men. Atherosclerosis, 70: 155–160.
Schwarz, K. (1965) Role of vitamin E, selenium and related factors in experimental nutritional liver diseases. Federation Proc. 24: 58–62.
Seamon, K. B. & Daly, J. W. (1986) Forskolin: Its biological and chemical properties. Adv. Cyclic Nucl. Protein Phosphoryl. Res. 20: 1–150.
Shamberger, R. J. (1983) Selenium Deficiency Diseases in Animals. In: Biochemistry of Selenium, pp. 31–58. Plenum Press, New York, NY.
Sunde, R. A. (1990) Molecular biology of selenoproteins. Annu. Rev. Nutr. 10: 451–474.
Sword, J. T., Pope, A. I. & Hoekstra, W. G. (1991) Endotoxin and lipid peroxidation in vitro in selenium- and vitaminE-deficient and adequate rat tissues. J. Nutr. 121: 258–264.
Tappel, A. L. (1965) Free radical lipid peroxidation damage and its inhibition by vitamin E and selenium. Federation Proc. 24: 73–79.
Tsen, C. C. & Tappel, A. L. (1958) Catalytic oxidation of glutathione and other sulfhydryl compounds by selenite. J. Biol. Chem. 233: 1230–1232.
Turan, B., Desilets, M., Acan, L. N., Hotomaroglu, Ö., Vannier, C. & Vassort, G. (1996) Oxidative effects of selenite on rat ventricular contractility and Ca movements. Cardiovasc.Res. 32: 351–361.
Turan, B., Hotomaroglu, O., Kilic, M. & Demirel-Yilmaz, E. (1999) Cardiac dysfunction induced by low and high diet antioxidant levels comparing selenium and vitamin E in rats. Regulatory Tox. Pharmacol. 29: 142–150.
Ugur, O. & Onaran, H. O. (1997) Allosteric equilibrium model explains steady-state coupling of beta-adrenergic receptors to adenylate cyclase in turkey erythrocyte membranes. Biochem. J. 323: 765–776.
Wang, Z., Bunce, G. E. & Hess, J. L.(1993) Selenite and Ca2+ homeostasis in the rat lens: effect on Ca-ATPase and passive Ca2+ –transport. Curr. Eye Res. 12: 2213–2218.
Wittenberg, B. A., White, R. L., Ginzberg, R. D. & Spray, D. C. (1986) Effect of calcium on the dissociation of the mature rat heart into individual and paired myocytes: Electrical properties of cell pairs. Circ. Res. 59: 143–150.
Wolf, B. B. & Harden, T. K. (1981) Guanine nucleotides modulate the affinity of antagonists at beta-adrenergic receptor. J. Cyclic Nucleotide Res. 7: 303–312.
Yang, G., Wang, S., Zhou, R. & Sun, S. (1983) Endemic selenium intoxication of humans in China. Am. J. Clin. Nutr. 37: 872–881.
Yang, F. Y. & Yang, M. Z. (1989) Reaction of Se with SH groups in spectrin is involved in the stabilization of erytrocyte membrane skeleton. Biochem. Int. 18: 1085–1091.
Young, J. D., Crowley, C & Tucker, E. M. (1981) Heamolysis of normal and glutathione-deficient sheep erythrocytes by selenite and tellurite. Biochem. Pharmacol. 30: 2527–2530.
Ytreus, K., Ringstad, J., Myklebust, R., Norheim, G. & Mjos, O. D. (1988) The selenium-deficient rat heart with special reference to tolerance against enzymatically generated oxygen radicals. Scand. J. Clin. Lab. Invest. 48: 289–295.

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