Title

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Effects of Krill-derived phospholipid-enriched n-3 fatty acids on Ca^{2+} regulation system in cerebral arteries from ovariectomized rats

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Aims: To investigate effects of n-3 polyunsaturated fatty acids on cerebral circulation, ovariectomized (OVX) rats were administered phospholipids in krill oil (KPL) or triglycerides in fish oil (FTG); effects on the Ca^{2+} regulating system in their basilar artery (BA) were then analyzed.

Main methods: The rats were divided into 4 groups: control, OVX, OVX given KPL (OVXP), and OVX given FTG (OVXT) orally, daily for 2 weeks**.** Time dependent relaxation (TDR) of contractile response to 5HT in BA was determined myographically, Na^{+}/Ca^{2+} exchanger (NCX) 1 mRNA expression was determined by real time PCR, and nucleotides were analyzed by HPLC.

Key findings: The level of TDR in OVX that was significantly lower than in the control, was inhibited by L-NAME and indomethacin**;** TEA inhibited TDR totally in the control but only partly in OVXP and OVXT. Relaxation induced by the addition of 5 mM **of** KCl to the BA pre-contracted with 5-HT was inhibited by TEA in the controls, OVXP and OVXT, but not in OVX. Overexpression of NCX1 mRNA in the BA from OVX was significantly inhibited by FTG. The ratio of ADP/ATP in cerebral arteries from OVX was significantly inhibited by KPL and FTG. Levels of triglyceride and arachidonic acid in the plasma of OVX increased, but were significantly inhibited by KPL and FTG.

Significance: Ovarian dysfunction affects Ca^{2+} activated-, ATP-sensitive- K^{+} channels and NCX1, which play crucial roles in the autoregulation of cerebral blood flow. Also,

KPL may become as good a supplement as FTG for postmenopausal women.

Keywords: Krill-derived phospholipids, eicosapentaenoic acid, docosahexaenoic acid, ovariectomized rats, cerebral artery, Na^{+}/Ca^{2+} exchanger, nucleotides metabolism

1. Introduction

Cerebrovascular dysfunction is more common in postmenopausal than premenopausal women, suggesting the vascular protective effects of estrogen [1, 2]. The actions of estrogen on the brain include increased cerebral blood flow [3], promotes endothelium-dependent relaxation by increasing nitric oxide, prostacyclin, and hyperpolarizing factor, and inhibits the mechanisms of vascular smooth muscle contraction including protein intracellular Ca^{2+}, protein kinase C [4] and lipid profile [5]. Because changes in cerebrovascular functions contribute to the pathogenesis of stroke [6], estrogen presents a potential treatment for cognitive decline in women with dementia syndromes, such as Alzheimer's disease [7]. A better understanding on the action of estrogen on cerebrovascular function holds promise for the development of new therapeutic entities that could be useful in preventing or treating a wide variety of cerebrovascular diseases. Controversy surrounding the use of hormone therapy for cardiovascular and neuronal health has contributed to the decline in its post-menopausal use [8, 9]. Many women now use alternative therapies for postmenopausal health including dietary soy and isoflavone supplements instead of, or in addition to,

traditional hormone therapy [10, 11]. Moreover, n-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been suggested as being involved in the development and maintenance of learning memory performance [12-16]. The n-3 PUFAs ameliorated endothelial dysfunction in diabetic rats [17], and led to attenuation of the contractile responses of isolated resistance arteries [18].

Fish oil typically contains n-3PUFAs in the form of triglycerides (TG) or as fatty acid ethyl esters [19]. Fish intake and ingestion of EPA, DHA and in some cases alpha-linolenic acid (ALA) have been associated with reduced risk of cardiovascular events and death [20]. Antarctic krill (*Euphausiasuperba*) is a shrimp-like crustacean rich in both EPA and DHA [21]. Knowledge of any interaction between n-3 PUFAs and estrogen would provide better strategies for the development and maintenance of brain circulation and learning memory. The contractile response of the intact basilar artery (BA) to 5-HT comprises of a phasic contraction followed by a time-dependent relaxation (TDR) [22]. The endothelium-dependent TDR includes nitric oxide (NO)- and cyclooxygenase-independent components, which is related to K^+ channel pathways and Na^+ pump activity in BA [22]. The effects of estrogen deficiency on TDR in cerebral circulation is not well understood, particularly with regard to the roles of K^+ channels and the Na^+/Ca^{2+} exchanger (NCX). The aim of this study was to determine whether a combined treatment with EPA and DHA, as provided by phospholipids in

krill oil (KPL) or by triglycerides in fish oil (FTG), has a beneficial effect on the Ca^{2+} regulating system in BA isolated from ovariectomized (OVX) rats.

2. Materials and Methods

2.1. Animals and diet

All rats were handled and killed in accordance with the procedures outlined in the Guidelines for Animal Experimentation of Shimane University, as compiled from the Guidelines for the Animal Experimentation of Japanese Association for Laboratory of Animal Science. Wistar rats purchased from CLEA Japan (Osaka, Japan) were housed in a room under the following controlled environmental conditions: 23 ± 2°C, 50 ± 10% relative humidity, 12 / 12 h light/dark cycle, 13-15 cycles of air exchange / h, and given food and water ad libitum. The animals were provided with a fish-oil-deficient diet (F-1 TM; Funabashi Farm, Funabashi, Japan). Inbred second-generation female rats fed the same F-1 diet were used in the study [21].

KPL obtained from Nippon Suisan Co. Ltd. (Tokyo, Japan) was weighed and emulsified daily in twice its volume with sterilized water. The fatty acid composition of the KPL emulsion was as described previously [21]. The rats were divided into 4 groups: Sham (Control), ovariectomized (OVX), OVX treated with KPL (182mg EPA + 118mg DHA; OVXP), and OVX treated with FTG (203mg EPA + 97mg DHA; OVXT). Control and OVX rats received sterilized water only. All the rats were provided with F-1 TM.

2.2. Blood sample preparation

The rats were anesthetized with diethyl ether and exsanguinated from the inferior vena cava with heparinized syringes. Blood samples were collected into polyethylene tubes and centrifuged for 20 min at 3.000rpm at 4°C to separate the platelet-poor plasma, as previous described [13].

2.3. Fatty acid composition of plasma and fat tissue

Analysis of fatty acid levels in plasma was carried out with a modified one-step reaction as reported by [23] and described previously [24]. Gas chromatograph separation was done on a Model 5890 I1 gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with a flame ionization detector and an automatic sampler (Model 7673).

2.4. Basilar artery ring preparation and organ bath setup

The rats were anesthetized with diethyl ether, the whole brain quickly removed, and the basilar artery (BA) isolated from the brain was gently cleaned of any connective tissue in Krebs Henseleit buffer (KHB), containing 118 mM NaCl, 4.5 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.5 mM $MgSO_4$, 25 mM $NaHCO_3$, and 11 mM glucose, pH 7.4, and bubbled with 95% O_2 / 5% CO_2, then cut into 2 ring preparations (each 2.5-3.0 mm long) under a dissecting microscope. The cleaned preparations were placed in an organ chamber (UFER, Medical Kishimoto, Japan) containing KHB at 37 ± 0.5°C. Two fine tungsten wires (Ø 50 µm) were then passed through the lumen of the basilar

artery, with one end of each wire connected to an isometric transducer (T7-8-240, ORIENTEC, Tokyo, Japan) and the other attached to the holder; the isometric tension was then recorded on a polygraph (RECTIGRAPH-8K, San-ei, Tokyo, Japan). The experiments were monitored by a computer-based analysis system in Mac-Lab and Chart 4.1 software (AD Instruments, Inc., Colorado Springs, Co, USA) as described previously [22].

2.5. Quantitative RT-PCR for NCX1 and TREK-1

Total mRNA from the rat basilar artery was prepared using an RNeasy kit (QIAGEN) according to the manufacturer's instructions. RNA (0.5 μg) was reverse transcribed to cDNA by QuantiTect® Reverse Transcrption (QIAGEN), and real-time PCR reactions were carried out with the use of QuantiTect®S YBR®Green PCR (QIAGEN) on a 7500 Fast Real-Time PCR System (Applied Biosystems). Gene specific primer sets were purchased from Sigma Genosys. GAPDH housekeeping genes were used as internal controls to normalize mRNA expression. The primers used were NCX1 forward: 5'-CTCACCATTATTCGAAGAGG-3', reverse: 5'-CCAGGTTTGAAGATCACAGT-3' [25]. TREK-1 forward: 5'-CCCCTCTTTGGTTTTCTACT-3', reverse: 5'-CGAGATGATACGAATCTTGG-3'; and GAPDH forward: 5'-AACGACCCCTTCATTGAC-3', reverse: 5'- TCCACGACATACTCAGCAC-3' [26].

2.6. Analysis of nucleotides in cerebral vascular tissue

Mixed cerebral arteries were homogenized with citrate buffer and centrifuged for 15min at 3.000rpm at 4°C. The supernatant was processed for the determination of ATP, ADP, AMP and adenosine by high performance liquid chromatography (HPLC) with fluorescence detection [24].

2.7. Drugs

N ω -nitro-l-arginine methyl ester hydrochloride (L-NAME), 5-Hydroxytryptamine (5-HT), tetraethylammonium chloride (TEA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The drugs dissolved in distilled water were stocked as 1–10 mM aliquots. All chemicals and materials of the highest grade available commercially were used at the following final concentrations: 5-HT (100 nM); TEA (1 mM), a non-selective Ca^{2+}-activated K^{+} channel (K_{Ca}) blocker; L-NAME (100 μ M), a non-selective NO synthase inhibitor; indomethacin (10 μ M), a non-selective cyclooxygenase inhibitor.

2.8. Statistical analysis

Results are expressed as means ± S.E.M. All parameters were analyzed by one-way ANOVA with Bonferroni multiple comparison student t tests. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Characteristics of experimental rats

Body and uterine weight characteristics of the 4 groups of rats are shown in Table 1. The mean body weight of the rats stood at 137.7 ± 4.7 g and was not significantly different among the 4 groups before ovariectomy. Two weeks after ovariectomy, however, it differed between the control and the OVX, OVXP, OVXT rats. Two weeks after the treatment of OVX rats with either KPL or FTG, their body weight was different from that of the control rats, but not different from OVXP and OVXT rats. The uterine weight of OVX rats decreased significantly compared with that of controls 4 weeks after ovariectomy. Plasma level of triglyceride (TG) at 4 weeks after ovariectomy increased significantly in OVX rats as compared with that in the controls; this increase was significantly inhibited by the dietary treatment with either KPL or FTG. Similarly, HDL-C decreased significantly in OVX rats compared with that in the controls; this change was partly inhibited by dietary treatment with either KPL in OVXP rats or FTG in OVXT rats. The non-HDL-C was not different among the 4 groups (Table 1).

The level of plasma estrogen in OVX rats showed a 35% decrease compared with that in the controls (data not shown).

3.2. Contractile responses of basilar artery to 5-HT

Contractile response of BA rings to 5-HT comprised an initial fast contraction (phasic component) followed by a sustained contraction (tonic component). The amplitude of the tonic component decreased gradually in a time-dependent manner in the 4 groups

(Fig. 1-A). In the BA with endothelium (intact), 5-HT-induced contraction was followed by profound relaxation; in the BA without endothelium (denuded), it was enhanced, while relaxation was almost absent The time-dependent decrease in the tonic component (called TDR) from the peak tension of the intact BA, which was significantly less in OVX rats than in controls, was inhibited in the BA from OVXP and OVXT rats (Fig.1-B). TDR in the BA from the four groups reached a similar level in the presence of L-NAME and indomethacin (Fig. 1-C); however, it was almost inhibited in the presence of L-NAME, indomethacin and TEA (a Ca^{2+}activated K^+ channel (K_{ca}) blocker); TDR in the BA from OVX was significantly different from that in OVXP (Fig. 1-D).) Without inhibitors, the level of TDR in the BA from OVX rats was lower than that in the control; it was inhibited, however, by L-NAME and indomethacin. TDR was less sensitive to TEA in the BA from OVXP rats.

3.3. Effects of KPL and FTG on KCl-induced relaxation

Relaxation induced by the addition of 5 mM of KCl to the BA precontracted with 100 nM of 5-HT was observed in all the preparations in the presence of L-NAME and indomethacin (Fig. 2-B). A transient relaxation produced by very low concentrations of K^+ (0 to 5 mM) is consistent with the hyperpolarization produced by the sodium-potassium pump ($Na^+/K^+{}_{ATPase}$). The relaxation was inhibited by TEA in the controls, OVXP and OVXT rats, but not in the BA from OVX rats (Fig. 2-C). The

relaxation in OVX rats was not affected by treatment with TEA.

3.4. Effects of KPL and FTG on the expression of NCX1 and TREK-1

Since the TDR in the BA is partly related to the Na^+ pump and NCX [22], we examined the expression of NCX1 mRNA in the BA from the 4 groups of rats (Fig.3-A). The expression NCX1 in the BA from OVX rats showed a 3-fold increase over that in the controls. This increase was significantly inhibited in OVXT rats by the dietary intake of FTG. The main PUFA target is the potassium channel, TREK-1, which belongs to the new family of two-pore domain potassium channels (K2P) and is known to be potently activated by PUFAs [27]. The expression of TREK-1 mRNA in OVX rats was not different from that in the control and OVXP rats, but it showed a downward tendency in OVXT rats (Fig.3-B).

3.5. Effects of KPL and FTG on nucleotide metabolism

To determine whether the nucleotide metabolism is related to the ATP-sensitive K^+ (K_{ATP}) channel and to the reduction in oxidative stress in cerebral arteries by the dietary intake of KPL or FTG, we measured nucleotides in the cerebral arteries from the 4 groups of rats. ATP showed a downward tendency in OVX, OVXP and OVXT rats as compared with that in the controls. On the other hand, the reduced ADP in OVX rats demonstrated an inhibitory tendency in OVXP rats by the dietary intake of KPL. AMP

and adenosine were not different among the 4 groups. The ADP/ATP ratio in the OVXP and OVXT rats increased 2-fold over that in OVX rats. Both KPL and FTG treatment significantly inhibited a decrease in the ADP/ATP ratio in OVX rats (Fig. 4).

3.6. Effects of KPL and FTG on fatty acid composition in the plasma and fat tissue

The fatty acid composition in the plasma changed after ovariectomy is shown in Table 2. The proportion of arachidonic acid (AA) was significantly higher and that of oleic acid (OLA) significantly lower in OVX rats as compared with that in control rats. The ratio of n-6 PUFA to n-3 PUFA (n-6/n-3) was also significantly higher in OVX rats than in the controls. Dietary intake of either KPL or FTG lowered the proportion of AA and the ratio of n-6/n-3 significantly in OVXP, OVXT and control rats as compared with OVX rats. By contrast, OLA in OVX rats was not influenced by the dietary intake of either KPL or FTG. In OVXP and OVXT rats, the proportions of EPA and DHA were significantly high due to the dietary intake of KPL and FTG, whereas the proportion of AA was significantly low by the same treatment. We also analyzed fatty acid composition of fat tissue as shown in Table 3.

4. Discussion

The results in this study indicated that consuming KPL or FTG containing the n-3 fatty acids, EPA and DHA, is associated with changes in the TDR of contractile response to

5-HT, especially in the expression of NCX1 in the BA isolated from OVX rats. Our study also demonstrated the contraction and metabolic pathways specified by estrogen deficiency, and suggested how interactions among such pathways determine the effects of the dietary intake of KPL and FTG on the cerebrovascular contractile system.

The level of TDR in OVX was lower than that in the control, and the difference was inhibited in the presence of L-NAME and indomethacin, suggesting that endothelium-dependent relaxation of BA is partly mediated by NO, either alone or in concert with a relaxant cyclooxygenase product. AA increased in plasma and fat tissue of OVX, and may thus be related to cyclooxygenase products. TEA totally inhibited the TDR in the control and OVX, but only partly in OVXP and OVXT (Fig.1). The K_{Ca} channels facilitate feedback regulation of the rise in intracellular Ca^{2+}, membrane depolarization and vasoconstriction. Since K_{Ca} channels are major determinants of vascular tone, their activation causes hyperpolarization of membrane potentials, which leads to the closing of voltage-gated Ca^{2+} currents and subsequent vasorelaxation [28]. Our results regarding the effects of TEA must be related to the significance of the function of K_{Ca} channels under cellular conditions. The TDR in OVXP was significantly less sensitive to TEA (Fig.1-D); therefore, another mechanism of the manifestation of TDR must be present; besides K_{Ca} channels.

DHA is an important modulator of vascular function, producing potent vasodilatation through the activation of K_{Ca} channels (especially BK channels) in vascular smooth

muscle cells by its epoxygenase metabolites that modulate intracellular Ca^{2+} homeostasis [29]. DHA has also been shown to inhibit numerous ion channels, such as the cardiac voltage-gated sodium currents, the L-type Ca^{2+} currents, the T-type Ca^{2+} currents, the delayed rectifier K^+ currents, and the transient outward K^+ currents [30, 31]. Thus, in our study, PUFAs in KPL and FTG could affect the TDR of contractile response to 5-HT in the BA from OVX rats.

We have previously reported that TDR is partly inhibited by ouabain, a Na^+/K^+-ATPase blocker [22]. Na^+/K^+-ATPase activation inhibits Ca^{2+} mobilization in endothelial cells and, thereby, causes endothelium-dependent relaxation [32]. The K^+-induced relaxation is produced by the activation of Na^+/K^+-ATPase in the intact BA [22]. The transient vasodilation produced by very low concentrations of K^+ (0 to 5 mM) is consistent with the hyperpolarization produced by Na^+/K^+-ATPase. Some studies have shown that the mechanism of EDHF is related to K^+, Na^+/K^+-ATPase and K_{IR} [33,34]. These factors might be two aspects of one regulating mechanism, but there is no evidence for this hypothesis. In this study, the K^+-induced relaxation in OVX rats was observed even in the presence of L-NAME, indomethacin and TEA (Fig.2-C). Most agonists including 5-HT stimulate Ca^{2+} influx from the extracellular space through voltage-gated, receptor-operated, and store-operated channels. Ca^{2+} homeostatic mechanisms tend to reduce 1) intracellular Ca^{2+} ($[Ca^{2+}]i$) by activating Ca^{2+} extrusion through the plasma membrane Ca^{2+} pump and the NCX, and 2) the uptake of excess Ca^{2+} by the sarcoplasmic

reticulum and possibly the mitochondria. NCX is often thought of as a Ca^{2+} extrusion pathway; however, depending on membrane potential, the transmembrane ionic gradients of Na^{+} and Ca^{2+}, and the relative importance of intracellular Ca^{2+}, NCX could contribute to either Ca^{2+} extrusion or Ca^{2+} influx (reverse-mode NCX) [35,36]. The NCX family comprises three isoforms, NCX1~NCX3, with NCX1 being the dominant one in arterial smooth muscles that regulates arterial tone and blood flow [35]. The higher expression of NCX1 mRNA in OVX rats is consistent with the notion that K-induced relaxation through the NCX system is related to the Na^{+} pump (Figs.2 and 3). This higher expression was significantly inhibited by the dietary intake of FTG. Nonetheless, KPL did not inhibit the higher expression in the BA of OVX rats. This different effect of KPL on NCX1 expression may relate to a signal transduction of phospholipids metabolism.

K_{ATP} channels contribute to the resting membrane conductance of some types of smooth muscles and open under situations of metabolic compromise [37, 38]. They are characteristically activated by declining concentrations of ATPi or elevated concentrations of ADP, followed by changes in the ratio of ADP/ATP; an increase in the ADP/ATP ratio opens K_{ATP} channels, leading to membrane hyperpolarization [39]. Thus, it is assumed that K_{ATP} channels provide a link between cell metabolism and membrane excitability [40]. In our study, alteration of the ADP/ATP ratio in OVX rats was significantly inhibited by the dietary intake of KPL and FTG (Fig.4). Our results showed that the effects of estrogen deficiency on the nucleotide metabolism acted

through K_{ATP} channels in the cerebral arteries from OVX rats. Too much a stretch is to relate a change in ADP/ATP ratio to K_{ATP} channels. The inhibition of this change by KPL and FTG may also be related to other factors.

TREK-1 K channels are important in vascular responses to PUFAs; alpha-linolenic acid injections increase cerebral blood flow and induce vasodilation of the BA but not of the carotid artery [26]. This channel activation elicits vasodilation that probably accounts for the increase in cerebral blood flow induced by PUFAs such as alpha-linolenic acid or DHA [26]. We also assessed the expression of TREK-1 mRNA in the BA (Fig. 3), but found no significant difference among the 4 groups of rats. Since one of the K (2P) channels, TREK-1, is resistant to the blocking effects of TEA [41], we concluded that TREK-1 in the BA was not activated by the dietary intake of KPL and FTG in the presence of this resistance.

It is well established that body mass and fat mass increase as a result of ovariectomy, as also observed in this study (Table1). Estrogen replacement therapy decreases fat accumulation and improves serum profiles [42, 43]. In OVX rats, the dietary intake of KPL and FTG did not inhibit an increase in body mass, but it inhibited an increase of plasma TG (Table 1). Plasma levels of EPA and DHA increased markedly, and AA levels decreased significantly by the dietary intake of KPL and FTG (Table 2). These results were consistent with previous reports on KPL and FTG [44, 45].

The krill-derived phospholipids used in this study consist primarily of

phosphatidylcholine (80% of total lipids) as described before [21]. There was no difference in plasma levels of EPA and DHA between OVXP and OVXT rats. Regardless of the type of oil, the rates of increase were 4.3~5 times higher in EPA and 1.6~1.7 times higher in DHA, respectively, compared with those in OVX rats (Table 2.). DHA did not increase in plasma as much as expected, suggesting that DHA is more easily absorbed by cell membranes than is EPA. The fatty acid composition of brain tissue is affected by fatty acids in the plasma [21, 46]. Recently, Schuchardt et al. [47] have demonstrated that EPA+DHA are absorbed more in treatment with KPL than with FTG. A direct comparison of KPL with FTG has revealed similar changes in plasma fatty acids: while FTG reduces blood pressure, KPL does not [48]. We also found similar effects of KPL and FTG on the TDR of contractile response of the BA to 5-HT and the nucleotide metabolism in cerebral arteries, although it differed in degree but not in kind. These phenomena might be related to the dosage and the term treated with KPL and FTG in this study. Future studies will have to investigate whether longer dietary treatment and higher dietary level of KPL and FTG.

Conclusions

Our findings suggest that ovary dysfunction influences NCX1 expression in BA which may be crucial in the regulation of cerebral blood flow. These findings provide valuable information regarding the mechanisms that govern gender-based differences in

cerebrovascular disease. Moreover, emerging research shows that n-3 PUFAs may have additional health effects with improved cerebral arterial contraction through NCX1, K_{ATP} channels and Kca channels in OVX rats. Krill may in future become as good a source of n-3 PUFAs as fish oil supplements for postmenopausal women.

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Figure legends

Fig. 1. Contractile responses to 5-HT of basilar artery in each group

A: Typical tracing of contractile responses to 100 nM 5-HT of basilar arteries with (intact) without endothelium (denuded).

B-D: Summary of the relaxation from the peak tension of basilar arteries isolated from the four experimental groups in the presence or the absence of inhibitors. Values are means ± SEM from 8 rats in each group. * , Significantly different from controls ; #, Significantly different from OVX rats. ($P<0.05$)

Fig. 2. Effects of KCl on the 5-HT-induced contraction.

A: Time-dependent relaxation induced by 5mM of KCl application. B: Time-dependent relaxation induced by 5mM of KCl application in the presence of L-NAME and indomethacin. C: Time-dependent relaxation induced by 5mM of KCl application in the presence of L-NAME, indomethacin and TEA. Values are means ± SEM from 5 rats in each experimental group. * , Significantly different from controls ; #, Significantly different from OVX rats. ($P<0.05$)

Fig. 3. Effects of KPL and TFG treatment on the expression of NCX1 mRNA and TREK-1 mRNA in basilar arteries from the four experimental groups.

A: Quantification of NCX1 mRNA expression by RT-PCR in basilar arteries from four experimental rats. NCX1 values were normalized to GAPDH. Values are means ±SEM from four preparations in each group. * Significantly different from Controls. (P<0.01); #, Significantly different from OVX rats. (P<0.01) B: Quantification of TREK-1 mRNA expression by RT-PCR in basilar arteries from four preparations in each group. Values were normalized to GAPDH.

Fig. 4. Effects of KPL and TFG treatment on nucleotide metabolism in the cerebral arteries isolated from the four experimental groups.

Values are means ± SEM from 5 preparations in each group.

*, Significantly different from controls (P<0.05). #, Significantly different from OVX rats (P<0.05).