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THE COUPLED TRANSPORT OF Ca^{2+}, K^+ AND H^+ IONS IN HUMAN LYMPHOCYTES

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K^+ and Ca^{2+} ions play an important role in many physiological processes of cells. Ca^{2+} ions as secondary messengers also control such central processes in lymphocytes as proliferation and differentiation and activity of many intracellular enzymes too [1, 2]. There are many facts about presence of Ca^{2+} dependent K^+ channel, activated by increase of intracellular Ca^{2+} concentration [3, 4, 5, 6, 7, 8].These results are obtained either by using fluorescent dyes, reacting to change the membrane potential (membranes are hyperpolarized by K^+ efflux from the cells), or by using $^{86}Rb^+$ or patch-clamp technique.

It is known that β-receptor[1] blocker propranonol activates Ca^{2+} dependent K^+ channel in erythrocytes [9, 10], whereas the effect of propranolol on Ca^{2+}-dependent K^+ channel in lymphocytes is not investigated. Transmembrane K^+/H^+ exchange in lymphocytes is not studied yet through the exchange of intracellular K^+ ions to extracellular volume [11], affecting in this way many functions of lymphocytes. The use of ionophore allows to model processes occurring on cell membranes and to reveal the mechanism of ion transport.

There are no data in literature about direct registration and coupling of Ca^{2+}, K^+ and H^+ ions fluxes across the lymphocyte membrane. Therefore we have tried to registrate Ca^{2+}-dependent K^+ efflux from lymphocytes, obtained from human peripheral blood and tonsils, by the ion selective electrodes and using Ca^{2+} ionophore A23187 and β-blocker propranolol. The influence of protonophore ClCCP transmembrane pH gradient on K^+ efflux from the lymphocytes also was studied.

Material and methods

Lymphocytes from donors peripheral blood and tonsils, obtained immediately after tonsillectomy, were isolated by Ficoll-verographin gradient centrifugation [12]. The RPMI-1640 medium was used as a

standard buffer. The lymphocytes were twice washed in medium without a buffer, containing 150 *mM* choline chloride and 0.1 *mM* KCl. pH of medium was 5.8-6.0. In some cases lymphocytes were washed in the same medium containing 1 *mM* Tris-HCl, with pH, ranging from 6.8 to 7.9 (low-buffered medium). The pellet of lymphocytes was resuspended and placed in incubation tube with 1 *ml* of medium, which was the same as the washing medium, the magnet mixer MM-3M was used to mix the suspension. The final concentration of lymphocytes was approximately 40-50 10^6 *lym/ml*. When Ca^{2+}-dependent K^+-efflux was investigated Ca^{2+} was added to the incubation medium. The changes of K^+ and H^+ concentrations were registered with the help of K^+ and H^+ selective electrodes combined in the same tubes and joined to recorders by means of pH-meters. The graduation of electrodes was performed by technique of standard additions, i. e. by adding KCl and HCl standard solutions. In the experiments A23187, protonophore ClCCP valinomycin ("Chemapol"), propranolol, Tris ("Serva"), choline chloride ("Sigma"), RPMI - 1640 ("Flow") were used.

Results and discussion

After incubation of lymphocytes from peripheral blood and tonsils in a tube with unbuffered choline chloride medium during 3-4 *min* medium pH increases and then the process slows down. pH of suspension is established in the range of 6.3-6.8. We suppose that the change of pH depends on the OH^- efflux CH^+ influx from the cells. To determine pH in the peripheral blood lymphocytes saponin was added into the tube with final concentration 0.01%, which caused cell lysis and equalized the intra- (pH_i) and extracellular (pH_{ex}) of lymphocyte suspension.

Since the lymphocytes were incubated in medium without buffer, pH in the medium became the same as pH in the cell. According to the results of 5 experiments, the intracellular pH was 7.0±0.5. So, after incubation of the cells during 3-4 *min* transmembrane ΔpH $\Delta pH = pH_{ex} - pH_{ci}$ was negative and varied within the limits of 0.2-0.7 pH units. Thus, the extracellular H^+ concentration exceeded the intracellular one.

It has been discovered that protonophore caused appearance of K^+/H^+ transmembrane redistribution in peripheral blood lymphocytes was investigated. In this case the medium was unbuffered. As it is shown in Fig. 1 crudial K^+ efflux and H^+ influx occur. The speed of K^+ efflux in this experiment was 1.1 *nmol* $K^+/min.\times 10^6$ cells (the value of speed has calculated as the average amount of K^+ flowing out of the cells during 15 *min*). Addition of K^+ transporter valinomycin after 15 *min* incubation of lymphocytes with protonophore caused supplementary small K^+ efflux and H^+ influx. According to the results of the experiments K^+/H^+ stoichiometry was 1.35-1.45 (n=5). Addition of saponin at the end of the experiment showed, that pH_i was equal to 6.7-6.9. This shows that H^+ ions influx into cells changes intracellular pH.

To reveal the role of pH in K^+ efflux incubation lymphocytes were washed and incubated in a low buffered medium with pH 7.5-7.9. The experiments showed, that in these cases also when the intracellular

concentration of H^+ ions exceeded extracellular one, protonophore induced K^+ efflux. But the speed of K^+ efflux was significantly lower. The change of medium pH was practically not observed (Fig 1.).

Apparently in this case K^+ ions flow out together with some anions. Additions of saponin after the experiment had been completed showed, that pH_i was 7.2-7.3 and 7.3-7.5 correspondingly. The same results of K^+/H^+ exchange were obtained when we studied the influence of protonophore ClCCP on tonsillar lymphocytes.

Thus the increase of proton permeability of lymphocytes from peripheral blood and from tonsils causes also activation of K^+ permeability and K^+ efflux according to concentration gradient. If negative transmembrane ΔpH takes place, K^+ mainly changes with H^+, whereas at positive pH symport of K^+ and anions, most probably Cl (main cytoplasmic anion) occurs.

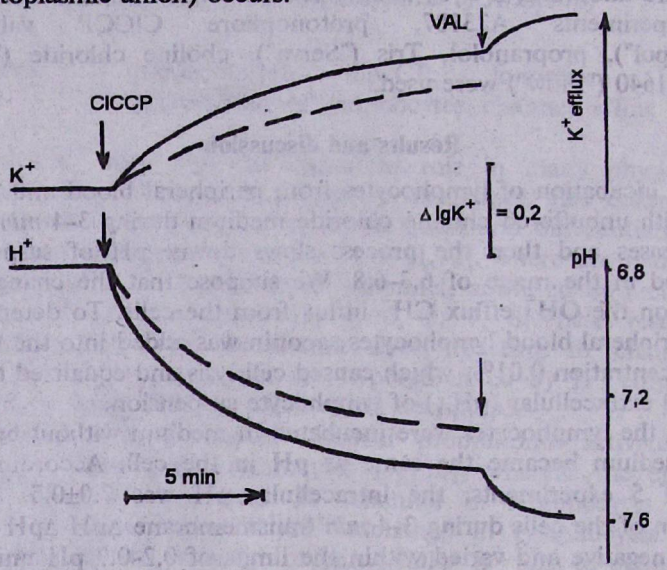

Fig. 1. Protonophore induced K^+ efflux and H^+ influx in peripheral blood lymphocytes. Incubation medium contains 150 *mM* of choline chloride and 0.1 *mM* of KCl. pH of suspension is 6.7. 18 μM of ClCCP and 1.2 μM of valinomycin are added. The touch line shows the influence of protonophore on K^+ efflux from lymphocytes, incubated in low-buffered suspension with pH 7.9 The final concentration of lymphocytes is $4x10^7/ml$.

We have investigated Ca^{2+}-dependent K^+ efflux at different pH, established in a tube after the lymphocytes addition. The experiments performed at suspension pH 6.3 (lymphocytes were added to unbuffered medium, containing 1*mM* of $CaCl_2$) showed that addition of ionophore A23187 did not cause K^+ efflux from lymphocytes either from peripheral blood, or from tonsils. Consequent addition of protonophore ClCCP caused expressed efflux and H^+ influx.

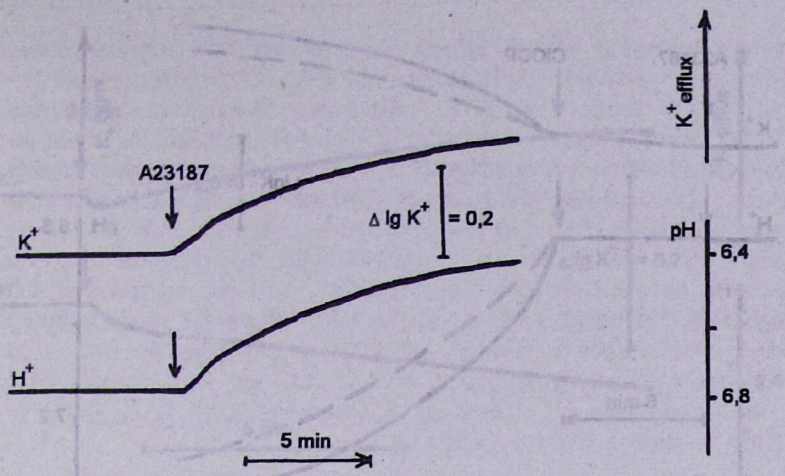

Fig. 2. The influence of A23187 on conjugated K^+ and H^+ efflux in blood lymphocytes. Unbuffered medium contains 1 *mM* of CaCL2. 5 *μM* of A23187 is added. pH of suspension is 6.8. Concentration of lymphocytes $4x10^7$/*ml*.

In Fig. 2 it is shown the action of A23187 on redistribution of the ions in the blood lymphocytes at suspension pH 6.8 (in that case also the lymphocytes were brought into unbuffered medium). Fig. 2 illustrates that A23187 causes K^+ efflux conditioned by Ca^{2+} entering into the cells and activating Ca^{2+}-dependent K^+ channel. In this case H^+ ions efflux from lymphocyte also takes place.

It is interesting to note that in blood lymphocytes, incubated in conditions when suspension pH is 7.2 to 7.4 (the lymphocytes were brought into low buffered medium, H^+ ions efflux is less expressed, although K^+ ions efflux is the same as at pH 6.8. The efflux of H^+ with K^+ ions, observed during the incubation of blood lymphocytes in unbuffered medium, may be caused by influx of Ca^{2+}. Besides, it is known that ionophore A23187 is a Ca^{2+}/H^+ antiporter [13]. The decrease of H^+ ions efflux from the lymphocytes, washed and incubated in low buffered medium, can be explained by the fact that pH_i was higher, than pH_i of "unbuffered". lymphocytes. Besides, presence of buffer obviously plays a definite role. The absence of K^+ ions efflux, observed at lymphocyte suspension pH_i 6.3 may be caused by sharp decrease at low pH of A23187-mediated Ca^{2+}/H^+ change from water to organic phases, which results in reduction of Ca^{2+} influx [13].

The experiments performed with the tonsillar lymphocytes showed that A23187 did not lead to the significant efflux of K^+ ions from the cells, even when suspension pH was 6.8 to 7.3. However the consequent addition of ClCCP caused K^+ ions efflux and crucial H^+ ions influx (Fig. 3).

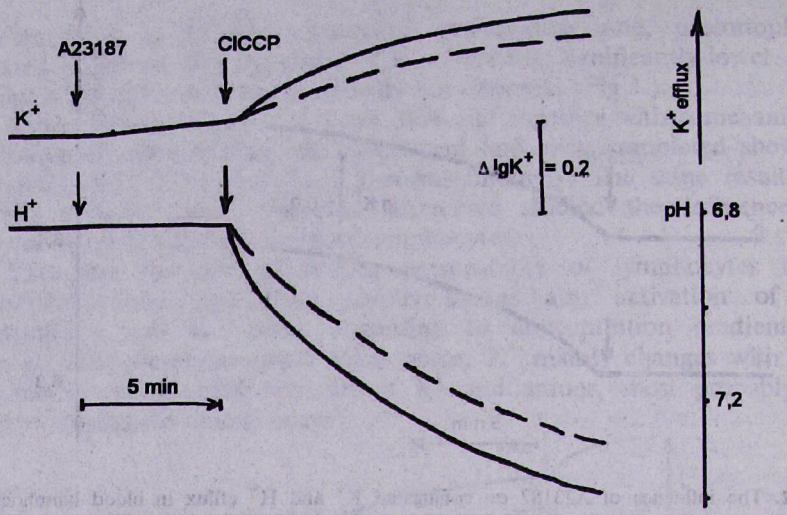

Fig 3. The influence of A23187 and ClCCP on redistribution of ions in tonsillar lymphocytes. Unbuffered medium contains 1 *mM* of $CaCl_2$. *5 μM* of A23187 and 18 *μM* of ClCCP are added. The touch line shows the influence of protonophore in absence of A213187. pH of suspension is 6.8. Concentration of lymphocytes is $5x10^7$ ml.

In case when protonophore was added without A23187, the efflux of K^+ ions was weak and change of pH was less expressed. This shows, that A23187 ionophore causes increase of K^+ permeability and as a result ClCCP inducted exchange of K^+ to H^+ ions increases.

It is interesting to note that whereas K^+ transporter valinomycin caused in blood lymphocytes significant efflux of K^+ ions, this effect in tonsillar lymphocytes was weakly expressed. On the analogy of the experiments with A23187, ClCCP addition to tonsillar lymphocytes, incubated with valinomycin, caused K^+ efflux activation and doubled the changes of pH in comparison with only protonophore addition.

It is known that in tonsillar lymphocytes population the amount of activated cells is much more than in blood lymphocytes [2]. It is possible that Ca^{2+}-dependent K^+ channels in tonsillar lymphocytes are initially activated, therefore Ca^{2+} ions transport into cells by A23187 ionophore causes only small additional activation of K^+ channel. Thus the action of A23187 ionophore and valinomycin in blood lymphocytes is expressed stronger, than in tonsillar lymphocytes.

Since, as we have noted above, propranolol activates Ca^+-dependent K^+ channel in erythrocytes, we had decided to examine its action on K^+ permeability of lymphocytes. In result of our experiments, it was revealed that propranolol in concentration 0.4 *μM* activates Ca^+-dependent K^+ efflux from blood lymphocytes, incubated in low-buffered choline chloride medium (Fig. 4).

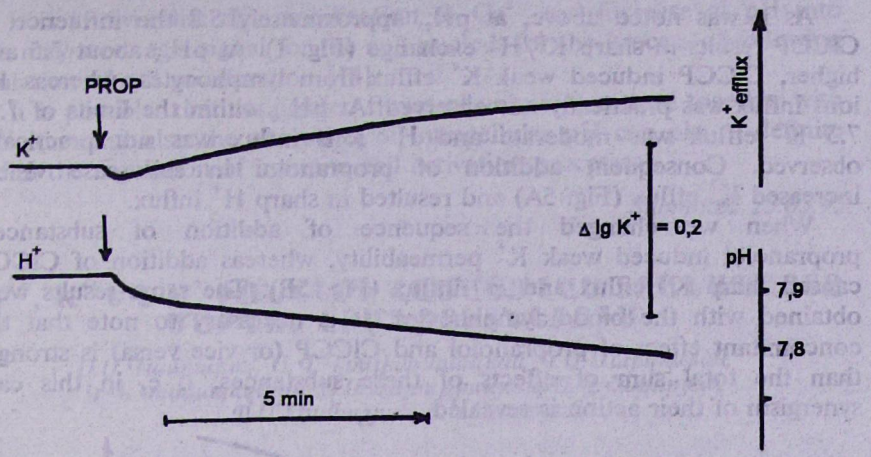

Fig. 4. The effect of propranolol on Ca^{2+}-dependent K^+ efflux from lymphocytes of peripheral blood, incubated in low buffered medium with pH 7.9. Medium contains 1 *mM* of $CaCl_2$ 0.4 *μM* of propranolol is added. Lymphocytes concentration is $4x10^7/ml$.

pH of medium after addition of lymphocytes was 8.0. It is necessary to note that at suspension pH 7.3-7.4 the effect of propranolol was less expressed. The same results were obtained during the study of the influence of propranolol on Ca^+ dependent K^+ channels of human tonsillar lymphocytes. This dependence of propranolol actions on pH may be explained by the fact that at the alkaline pH the quantity of nondissociating forms of propranolol is higher and this form permeates better through the membrane. Therefore Ca^+ influx should increase.

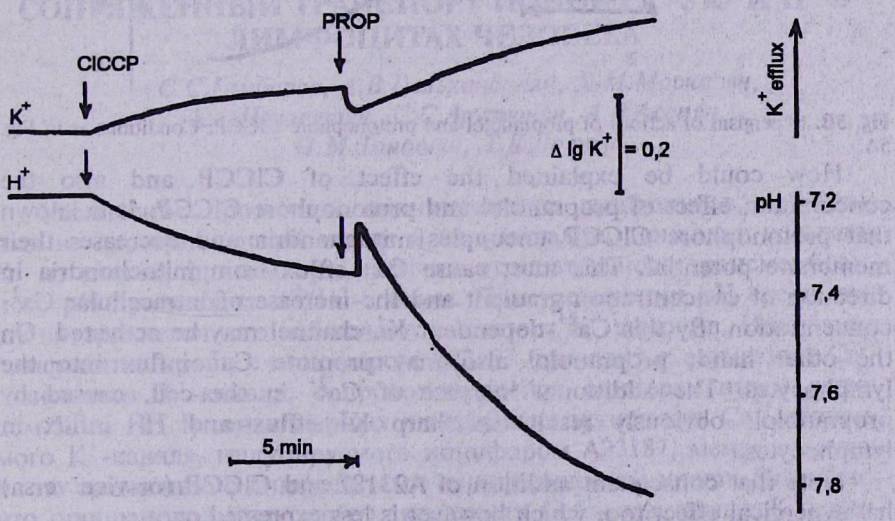

Fig 5A. Synergism of action of ClCCP and propranolol on Ca^{2+}- dependent K^+ efflux from tonsillar lymphocytes, incubated in low buffered medium with pH 7.2. Medium contains 1 *mM* of $CaCl_2$. 18 *μM* of ClCCP and 0.4 *μM* of propranolol are added. Concentration of lymphocytes is $10^7/ml$.

As it was noted above, at pH_{ex} approximately 5.8 the influence of ClCCP results in sharp K^+/H^+ exchange (Fig. 1). At pH_{ex} about 7.5 and higher, ClCCP induced weak K^+ efflux from lymphocytes, whereas H^+ ions influx was practically not observed. At pH_{ex} within the limits of 7.1-7.3 K^+ efflux was moderate and H^+ ions influx was not practically observed. Consequent addition of propranolol in this case visibly increased K^+ efflux (Fig. 5A) and resulted in sharp H^+ influx.

When we changed the sequence of addition of substances, propranolol induced weak K^+ permeability, whereas addition of ClCCP caused sharp K^+ efflux and H^+ influx (Fig. 5B). The same results were obtained with the blood lymphocytes. It is necessary to note that the concomitant effect of propranolol and ClCCP (or vice versa) is stronger than the total sum of effects of these substances, i. e. in this case synergism of their action is revealed.

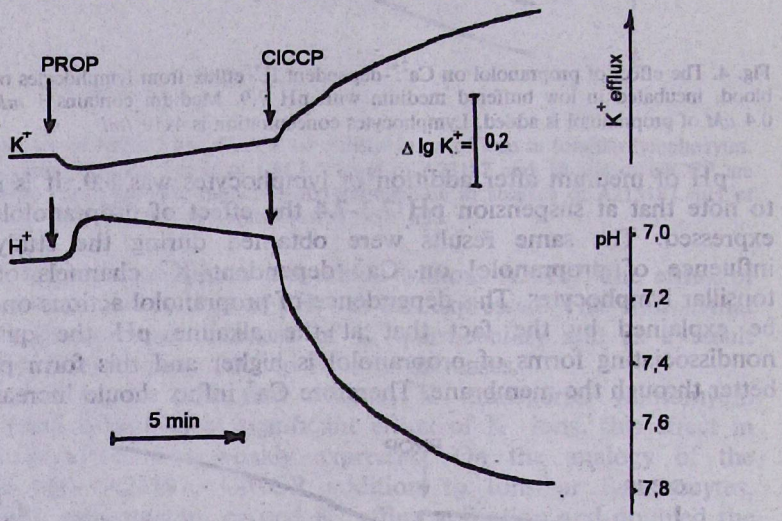

Fig. 5B. Synergism of actions of propranolol and protonophore ClCCP. Conditions as in Fig. 5A.

How could be explained the effect of ClCCP and also the concomitant effect of propranolol and protonophore ClCCP. It is known that protonophore ClCCP uncouples mitochondria and decreases their membrane potential. This must cause Ca^+ efflux from mitochondria in direction of concentration gradient and the increase of intracellular Ca^{2+} concentration. By this Ca^{2+}-dependent K^+ channel may be activated. On the other hand, propranolol also may promote Ca^+ influx into the lymphocytes. The additional increase of Ca^{2+} in the cell, caused by propranolol, obviously results in sharp K^+ efflux and H^+ influx in lymphocytes.

Note that consequent addition of A23187 and ClCCP (or vice versa) has synergical effect too, which however is less expressed.

It is known that the mobilization of Ca^{2+} and increase of pH into cells may serve as a signal for the cell nucleus for the process of following regulation of cell proliferation [14].

It is possible, that conjugated fluxes of univalent cations together with Ca^{2+} ions and also the alteration of intracellular pH can play a definite role in the regulation of immune cell activation processes.

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Ca^{2+}, K^+ ԵՎ H^+ ԻՈՆՆԵՐԻ ՀԱՄԱԿՑՎԱԾ ՏՐԱՆՍՊՈՐՏԸ ՄԱՐԴՈՒ ԼԻՄՖՈՑԻՏՆԵՐՈՒՄ

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Հայտնաբերված է, որ ClCCP պրոտոնոֆորը մարդու արյան եւ տոնզիլյար լիմֆոցիտներում առաջացնում է K^+-ի թափանցելիության մեծացում: Երբ միջավայրի pH-ը ցածր է ներբջջային pH-ից, K^+-ի ելքը տեղի է ունենում մեծամասամբ H^{2+}-ի հետ փոխանակման հաշվին, իսկ երբ միջավայրի pH-ը բարձր է ներբջջային pH-ից, ապա տեղի է ունենում K^+-ի եւ անիոնների սիմպորտ: A23187 իոնոֆորը ակտիվացնում է Ca^{2+}-ից կախված K^+ եւ H^+ անցուղիները, որն ուղեկցվում է H^+ իոնների միաժամանակյա ելքով: Ի տարբերություն ծայրամասային արյան լիմֆոցիտների, տոնզիլյար լիմֆոցիտներում Ca^{2+}-ից կախված K^+ անցուղիների ակտիվությունը թույլ է արտահայտված:

Ցույց է տրված,որ մարդու ծայրամասային արյան եւ նշագեղձերի լիմֆոցիտներում պրոպրանոլոլը ակտիվացնում է Ca^{2+}-ից կախված K^{2+} անցուղիները: Պրոպրանոլոլի եւ ClCCP համակցված ազդեցությունը K^+ իոնների ելքի վրա բնութագրվում է սիներգիզմով:

СОПРЯЖЕННЫЙ ТРАНСПОРТ ИОНОВ Ca^{2+}, K^+ И H^+ В ЛИМФОЦИТАХ ЧЕЛОВЕКА

С.С.Гамбаров, А.В.Гюльханданян, К.М.Маркосян, К.Г.Нагапетян, С.С.Арутюнян, А.В.Акопян, П.М.Танашян, Т.К.Давтян

Увеличение протонной проницаемости лимфоцитов, выделенных из периферической крови и из миндалин, приводит к выходу K^+ из клеток, причем при наличии отрицательного трансмембранного pH происходит K^+/H^+- обмен. При увеличении pH среды выше внутриклеточного наблюдается симпорт K^+ анионов. Инкубация лимфоцитов крови с ионофором A23187 приводит к активации Ca^{2+} зависимого K^+-канала, сопровождающейся одновременным выходом K^+ и H^+. В тонзиллярных лимфоцитах активность Ca^{2+}-зависимого K^+-канала, индуцируемого ионофором A23187, менее выражена по сравнению с лимфоцитами периферической крови. Показано, что пропранолол индуцирует активность Ca^{2+}- зависимых K^+-каналов в лимфоцитах периферической крови и небных миндалин человека. Совместное действие пропранолола и ClCCP на выход K^+ из

лимфоцитов независимо от последовательности их добавления характеризуется синергизмом.

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