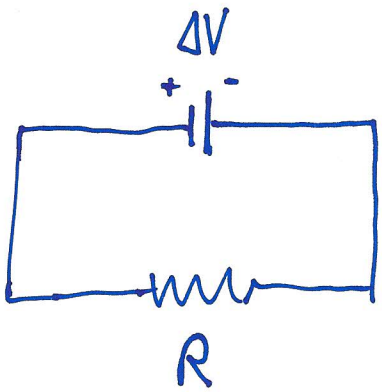


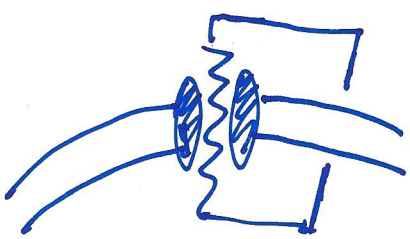
$$I = \frac{\text{cariche}}{A \cdot t} \quad (\text{DENSITÀ})$$

$$I = \frac{1}{R}\Delta V$$

$$\frac{1}{R} = G$$

R = Resistenza

G = Conduttanza



$$I = GV$$

se $V = 0 \Rightarrow I = 0$

NO nelle cellule!

se $V_m = 0$

il K^+ esce dalla cellula per ΔG

Na^+ entra nella " " "

(ELETTRODIFFUSIONE)

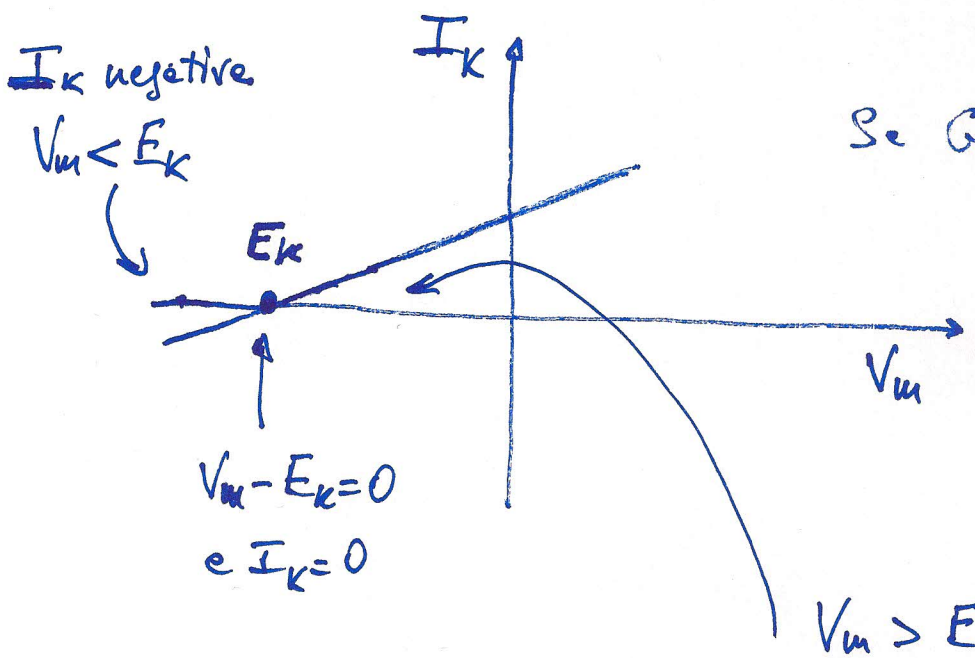
(I POSITIVA: CATIONI CHE ESCONO DALLA CELLULA
I NEGATIVA: " " ENTRANO NELLA CELLULA)

$$I_i = G_i \left(V_m - E_i \right) \quad \text{se } V_m = E_i \Rightarrow I_i = 0$$

per ora supponiamo di non considerare tipi specifici di canale, ma solo la permeazione di un certo ione.

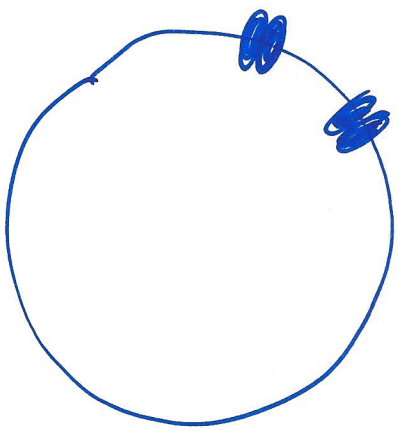
P.es.:

$$I_K = G_K \left(V_m - E_K \right)$$



Se G_K è COSTANTE

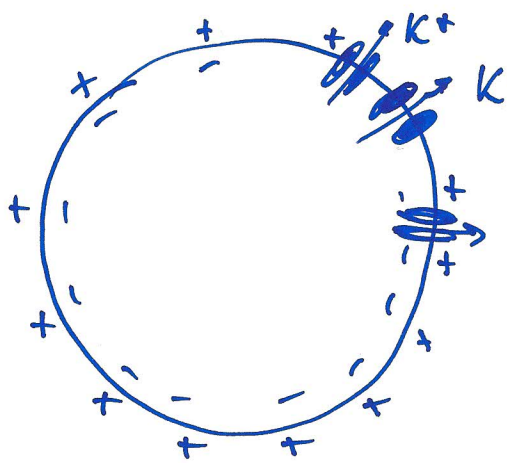
$t = 0$



CANALI TUTTI CHIUSI

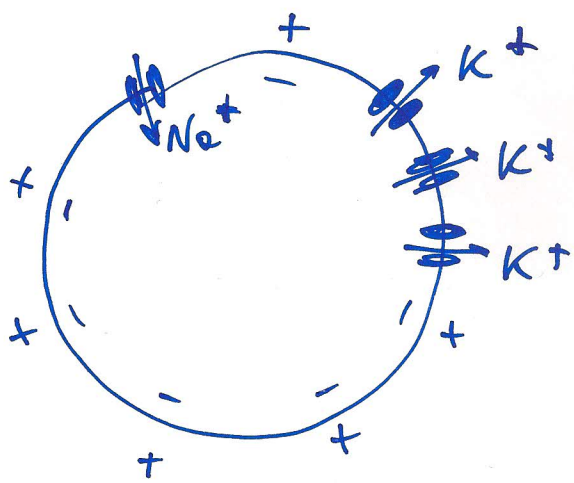
$V_m = 0$

$t = 1$ APRIAMO SOLO CANALI PER IL K^+



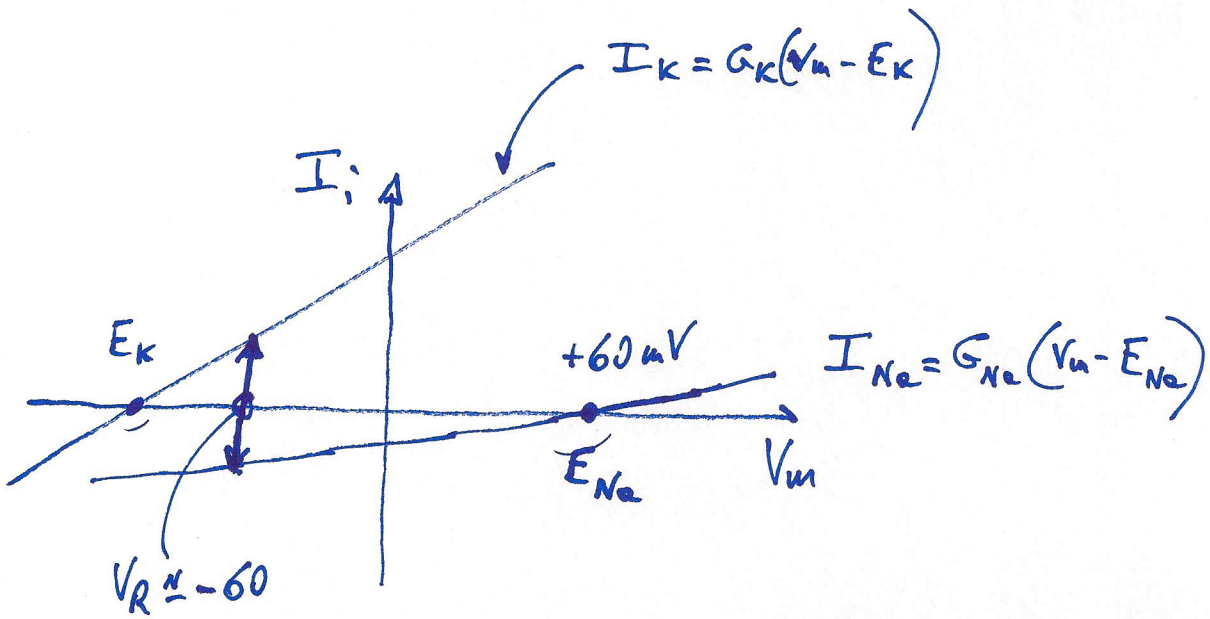
$V_m = E_K \simeq -90$ mV

$t = 2$ APRIAMO ANCHE UN PO' DI CANALI PER Na^+

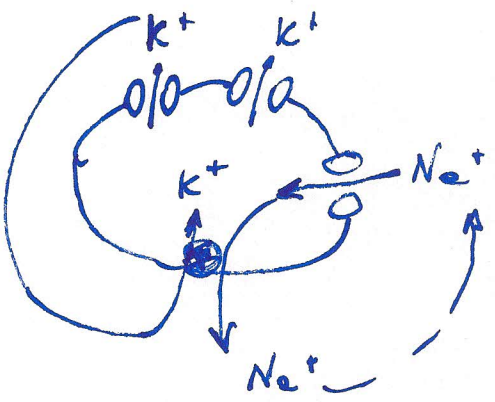


V_m diventa meno negativo (depolarizza) fino a raggiungere uno STATO STAZIONARIO (il V_R)

INTRODUZIONE AL V_{REST} (V_R)



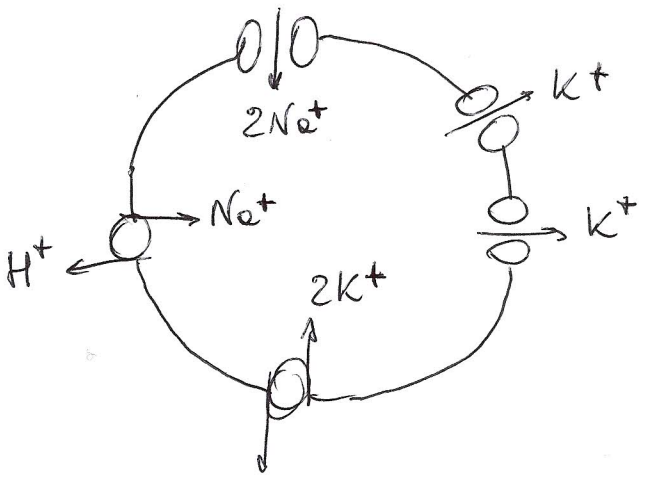
$$I_K + I_{Na} = 0$$



SIAMO AL $V_R \neq E_K$
$\neq E_{Na}$

LA CELLULA CONSUMA ATP
per mantenere V_R

PERCHÉ LA POMPA HA STECHIOMETRIA 3:2 ?



$3Na_{in} = 3Na_o$

$2K_{in} = 2K_o$

e $1H^+$?

P. es. bilancio (come cerca) di $1 HCO_3^-$ che esce.

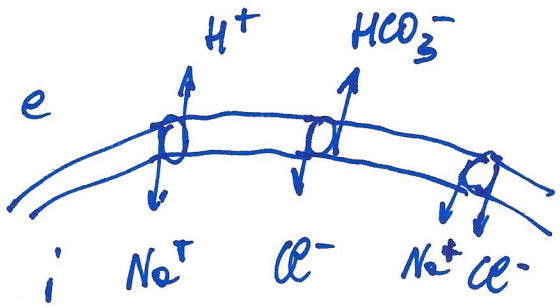
la cellula ha perso H_2CO_3

$$I_K + I_{Na} + I_{Cl} = 0 \quad \text{al } V \text{ di riposo}$$

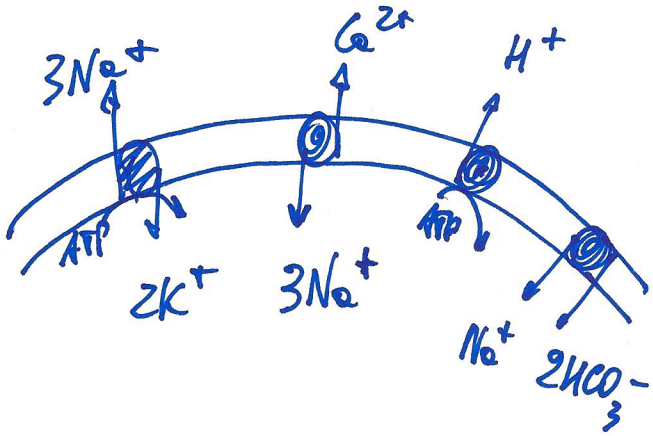
$$G_K (V_m - E_K) + G_{Na} (V_m - E_{Na}) + G_{Cl} (V_m - E_{Cl}) = 0$$

$$V_m = V_R = \frac{G_K E_K + G_{Na} E_{Na} + G_{Cl} E_{Cl}}{G_K + G_{Na} + G_{Cl}}$$

TRASPORTATORI ELETTRONEUTRI



TRASPORTATORI ELETTROGENICI



CONTRIBUTO DELLA POMPA DEL Na^+ AL V_{REST}.

I_i = corrente passiva per lo ione i

I_{ip} = corrente di pompa " "

r = rapporto Na^+/K^+ pompati

Allo stato stazionario: $I_i + I_{ip} = 0$

Cioè:

$$\left.\begin{aligned} I_{Na} + I_{NaP} &= 0 \\ I_K + I_{KP} &= 0 \\ r\,I_{KP} + I_{NaP} &= 0 \end{aligned}\right\} \text{SISTEMA}$$

$$r\,I_K + I_{Na} = 0$$

Introducendo $I_K = g_K (V_m - E_K)$ e $I_{Na} = g_{Na}(V_m - E_{Na})$,

si ricava

$$V_R = \frac{r g_K E_K + g_{Na} E_{Na}}{r g_K + g_{Na}}$$

ANALOGA A (MULLINS-NODA) → RICAVATA CON GHK

ESEMPIO DEL CONTRIBUTO DELLA POMPA Na/K AL V_R

1) SENZA POMPA: $r = 1$, $g_{Na}/g_K = 0.03$

$$V_R = \frac{g_K E_K + g_{Na} E_{Na}}{g_K + g_{Na}} = \frac{E_K + g_{Na}/g_K E_{Na}}{1 + g_{Na}/g_K} =$$

$$= \frac{-90 + 0.03(+60)}{1 + 0.03} \simeq -85.6 \text{ mV}$$

2) CON LA POMPA: $r = 1.5$, $g_{Na}/g_K = 0.03$

$$V_R = \frac{r E_K + g_{Na}/g_K E_{Na}}{r + g_{Na}/g_K} = \frac{1.5(-90) + 0.03(+60)}{1.5 + 0.03} =$$

$$\simeq -89.4 \text{ mV}$$

In questo caso il contributo è circa 4 mV (4.5% di V_R); si può arrivare fino al 15%, in certe cellule di mammifero.

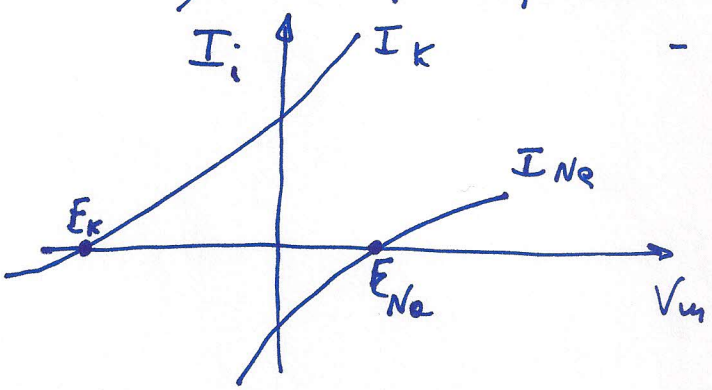
MODELLO TEORICO di GOLDMAN (N 1947)
PER RICAVARE LA FORMA DELLE I_i e V_R

Se V_R: $I_K + I_{Na} + \dots (p.es.\ I_{Cl}) \dots = 0$

Ma che forma hanno I_K e I_{Na}?

a) Si può usare $I_i = G_i (V - E_i)$, ricavando la forma di G_i sperimentalmente

b) Si può derivare una funzione che dia I_i in funzione di V_m, in base alle leggi dell'elettrodiffusione:
È l'equazione di GOLDMAN per la corrente (di un certo ione i) che ha questa forma:



ASSUNZIONI:
- CAMPO (ELETTRICO TRANSMEMBRANA) COSTANTE
- IONI CON FLUSSI INDIPENDENTI
- NON SI TIENE CONTO DELLE POSSIBILI VARIAZIONI del N di canali o del loro "GATING" (non si sapeva che esistessero canali ionici nel 1947).

Ponendo $I_{Na} + I_K + \dots I_{altre} = 0$ si può ricavare V_R.

EQUAZIONE DI GOLDMAN PER IL V_m DI RIPOSO

$$V_R = \frac{RT}{F} \ln \frac{P_K [K^+]_o + P_{Na} [Na^+]_o + P_{Cl} [Cl^-]_i + \dots}{P_K [K^+]_i + P_{Na} [Na^+]_i + P_{Cl} [Cl^-]_o + \dots}$$

Spesso nominata Equazione di Goldman-Hodgkin-Katz (GHK).

Bibliografia per canali K_{2P} e K_{IR}.

Renigunta V. *et al*.

Much more than a leak: structure and function of K_{2P} channels.

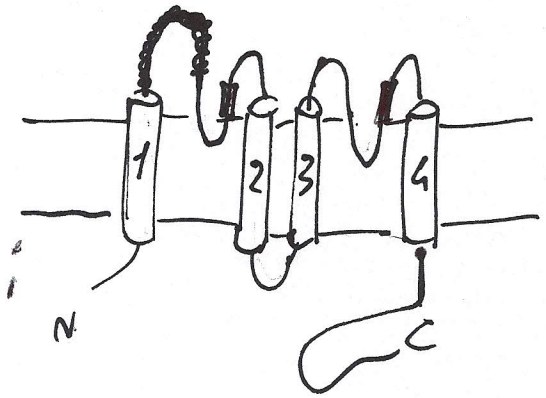
Pflügers Archiv 467: 867-894, 2015.

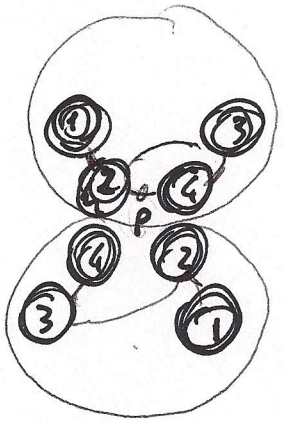
Sepulveda F.V. *et al*.

Molecular aspects of structure, gating, and physiology of pH-sensitive background K_{2P} and K_{IR} K^+-transport channels.

Physiological Reviews 95: 179-217, 2015.

K_{2P} S1 - S4
TM1 - TM4

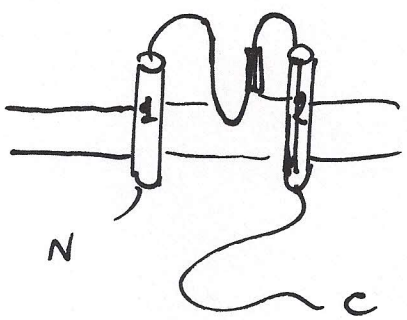


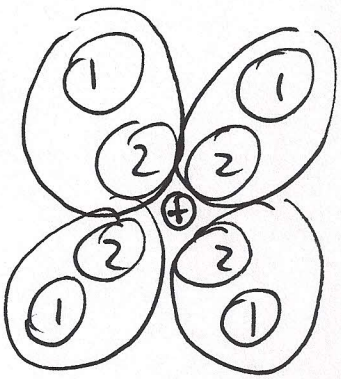


(X-ray)

SID = self-interacting domain (S-S with the one on the other subunit)

K_{ir}





K_{IR} = inward rectifiers
rectification entrante

amounts of truncated TREK-2 channels due to leaky scanning of the mRNA [251]. Thus, the combination of alternative splicing at the C-terminus and ATI can produce a large variety of protein isoforms.

Since the relative amount of ATI appears to vary between different tissues, any alteration of the biophysical properties of truncated TREK-1 channels may be functionally relevant. The first studies of the biophysical properties of TREK-1 channels in heterologous expression systems reported a single-channel conductance of 95–130 pS at positive potentials [111, 122, 206]. However, a later study showed that both in native rat cardiomyocytes and in heterologous expression systems TREK-1 channels with two different conductances were observed: one with ~40 pS and one with ~130 pS [156], as illustrated in Fig. 1a, b. The low-conductance channel was found to be more abundant, and in some cases, sudden transitions between the two conductances were found. For lack of an alternative explanation, the two different conductances were interpreted as two gating modes of the same channel [156]. With hindsight, it appears likely that the two different conductances were attributable to ATI; the observed sudden transitions between the two conductances [156] may have been coincidental. Thus, ATI of TREK-1 may indeed occur in the heart. In magnocellular neurosecretory cells of the hypothalamus, too, two different channels with TREK-1-like properties were found, and again the low-conductance channel ('a novel TREK-like channel') was found to be more abundant [111]. This might also be attributable to ATI.

In conclusion, ATI can produce TREK channels with different properties both in native cells and in heterologous expression systems. The reason for the differences in single-channel conductance and the physiological relevance of having channels with different conductances in the same cells (or in different cells) remains to be established.

The structure of K_{2P}-channels

Beginning in 2012 [43, 181], there has been enormous progress in our understanding of the structure of K_{2P}-channels. Since this is relevant for analysing the many functions of K_{2P}-channels described in this special issue of Pflügers Archiv, we here provide a very simple overview of the main structural features of K_{2P}-channels (Fig. 4).

Basic properties

The crystal structure of two K_{2P}-channels, TWIK-1 and TRAAK, has been solved by Miller and Long [181] and Brohawn et al. [43]. Most of the structural characteristics of TWIK-1 and TRAAK were found to be similar: The channels have an extracellular cap (consisting of two cap helices, C1 and C2) that is unique among ion channel structures (Fig. 4a).

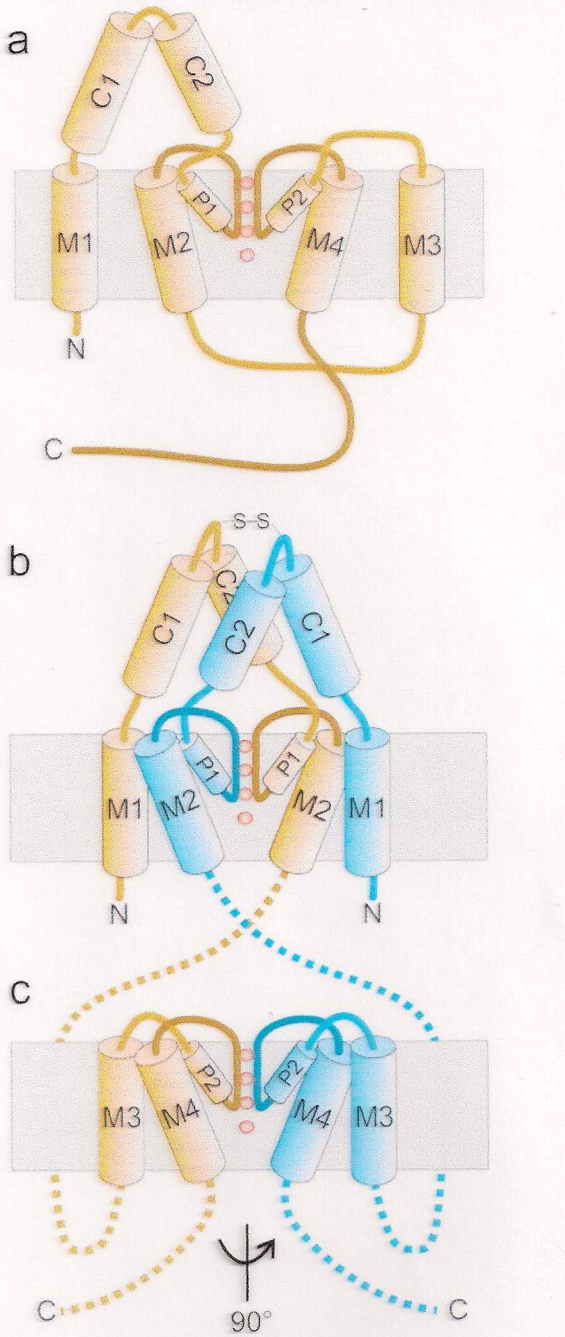


Fig. 4 The structure of K_{2P}-channels. **a** Topology of K_{2P}-channels. **b** Sketch of the structure of the N-terminal part of the two subunits (including the M1, C1, C2, P1 and M2 domains). **c** Sketch of the structure of the C-terminal part of the two subunits (including the M3, P2 and M4 domains). The helices are not drawn to scale. For clarity, the pore helices are relatively small

The cap extends ~35 Å above the extracellular membrane and covers an extracellular vestibule that has two lateral portals for K^+ ions. The pore-lining helices, M2 and M4, run obliquely through the cell membrane, whereas the outer helices, M1 and M3, are more vertically oriented. These basic properties may apply to all K_{2P}-channels. Both groups found that the

K2P SUBGROUPS BASED ON SEQUENCE HOMOLOGY

TWIK (K2P 1.1, 6.1, 7.1)
2P weakly inward rectifiers
background channels, TWIK-1 can convert to a non-selective cation channel

TREK (K2P 2.1, 10.1, 4.1)
TWIK-RELATED
Lipid and mechanosensitive
thalamus, immune cells, heart, adrenal etc

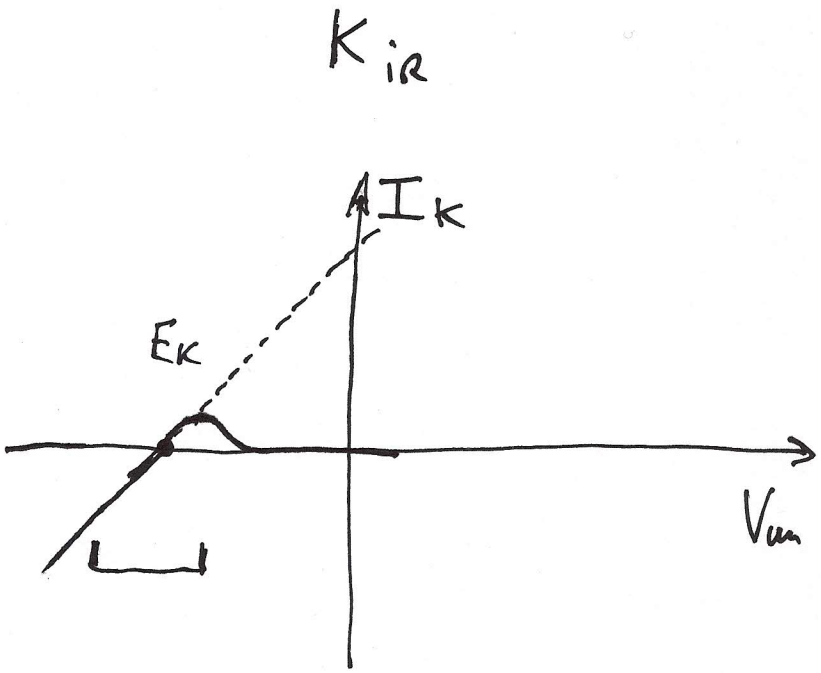
TASK (K2P 3.1, 9.1, 15.1)
TWIK-related ACID pH SENSITIVE
(extracellular pH 6.8-7.5): hypoxia/ischemia, epileptic seizures, leukocyte activation, bone reabsorption, synaptic clefts, T-tubules in muscle.

TALK (K2P 5.1 = TASK-2; K2P 16.1, 17.1)
ALKALINE pH-ACTIVATED
(inhibited by extrac. acidification but with pK much more alkaline).
pancreas (secretion HCO_3^-?), kidney

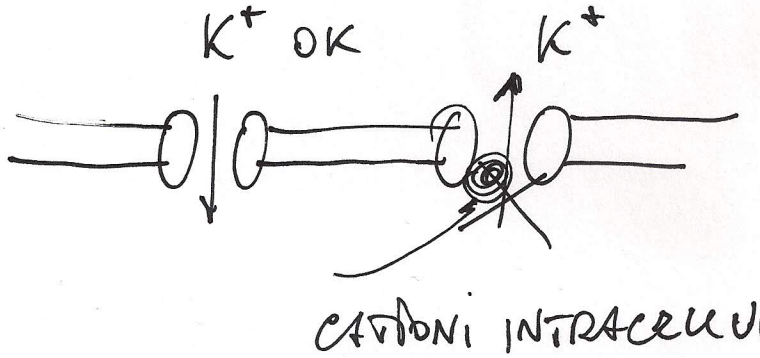
THIK (K2P 13.1, 12.1)
HALOTHANE-INHIBITED
ubiquitous, high expression in kidney, but also CNS.

TRESK (K2P 18.1)
SPINAL CORD K2P
high expression in sensory neurons (DRG and trigeminal ganglion, nociception)
the only activated by ↑$[Ca^{2+}]_i$

IR = RETTIFICATORI ENTRANTI (INWARD RECTIFIERS)
la I_K FLUISCE PIÙ FACILMENTE IN ENTRATA che in USCITA.

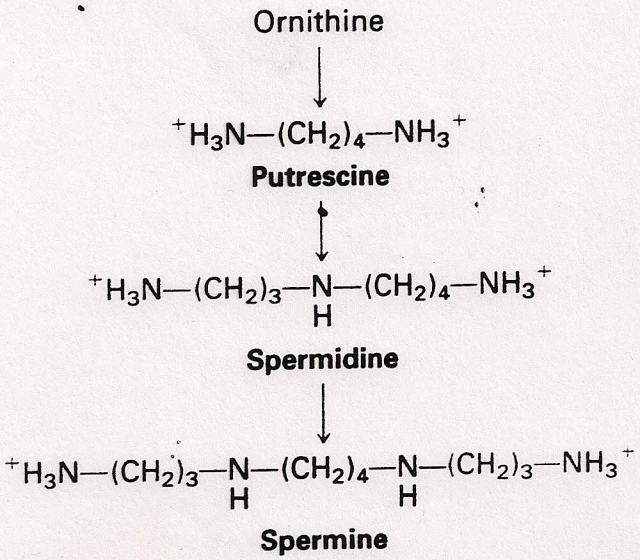


molto espressi p.es. negli ASTROCITI



CATIONI INTRACELLULARI

CATIONI CHE BLOCCANO I_{KIR} DAL LATO INTRACELLULARE



50-100 μM intracellulare

+ Mg^{2+}

Kir 2.1 — skeletal muscle
2.2 — cardiac " (I_{K1})

Kir 4.1 — main I_K in astrocytes

4.1 — brain, kidney†, stomach, sensory organs
4.2 — " " † liver, pancreas, lung
5.1* — " " , sensory organs

* probably a modulatory subunit

generally high sensitivity to extracellular Ba^{2+} and Cs^+ (but not Kir 2.1)
(differently from K_{2P})

generally inhibited by rel's pH intrac.

† basolateral, Kir 1.1 apical

$$I_i = \sum I_i \text{ (canali di ioni diversi)}$$

p.es. per il K^+:

$$I_K = G_K (V_m - E_K)$$

$$= \underbrace{G_{K2P}}(V_m - E_K) + \underbrace{G_{K1R}}(V_m - E_K) + \text{+ ALTRI (p.es. voltaggio-dipendenti)}$$