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Chemical names

ICI164384: *N-n*-butyl-11-(3,17β-dihydroxyestra-1,3,5(10)-trien-7α-yl)-*N*-methylundecanamide
ICI182780: 7α-[9-[(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonyl]estra-1,3,5(10)-triene-3,17β-diol

A role for neuronal K_{ATP} channels in metabolic control of the seizure gate

Birgit Liss and Jochen Roeper

ATP-sensitive K^+ (K_{ATP}) channels are expressed in many different tissues including the brain, where they couple energy metabolism to cellular excitability. Although their classical role in insulin secretion in pancreatic β-cells is well understood, their neuronal function remains unclear. Now, an important study using knockout mice provides clear evidence that neuronal K_{ATP} channels are crucial players in counteracting seizure induction and propagation.

K^+-selective ion channels belong to the superfamily of transmembrane proteins that couple a large diversity of physicochemical stimuli to the activity of their ion-conducting pores. These stimuli are predominantly transmembrane voltage gradients or the binding of a variety of extracellular and intracellular ligands. A large number of different genes encoding the principal pore-containing α-subunits and the regulatory β-subunits of K^+ channels have been identified[1], consistent with the functional diversity of K^+ channels. Indeed, two very different types of subunit team up to build ATP-sensitive K^+ (K_{ATP}) channels: four pore-forming subunits of the inwardly rectifying K^+ channel class, named Kir6.1 or Kir6.2, join together with four large β-subunits, the sulfonylurea receptors (SUR1 or SUR2A/B), which as members of the ABC (ATP binding cassette) transporter family possess two highly conserved nucleotide binding sites. Different combinations of these K_{ATP} channel subunits give rise to functional channels with distinct biophysical, pharmacological and metabolic properties[2–4].

The unique makeup of K_{ATP} channels allows them to convert the energy state of a cell (mainly expressed by the ATP:ADP ratio) directly into altered K^+ fluxes through the plasma membrane: the open probability of these channels is increased with decreasing energy levels, thus adapting excitability to energy metabolism. The close coupling between cellular activity and metabolic state might be of particular significance in neurones because they expend the majority of their energy on ion pumps such as the Na^+–K^+-ATPase to maintain the electrochemical gradients that are perturbed by electrical signalling.

Physiological function of neuronal K_{ATP} channels

The functional role of K_{ATP} channels is best understood for pancreatic β-cells,

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where they couple blood glucose concentrations to insulin secretion[5]. A similar role in sensing central glucose levels and triggering pancreatic glucagon secretion has recently been demonstrated for K_{ATP} channels in hypothalamic glucose responsive neurones[6]. However, K_{ATP} channels are expressed in many regions of the brain that are not involved in specific neuroendocrine functions such as glucose sensing. Therefore, a second, more general role for these channels has been proposed, in particular for excitable tissues such as muscle and neurones: the opening of K_{ATP} channels in response to a decreased ATP:ADP ratio hyperpolarizes the neurone, thus reducing or even completely abolishing electrical activity. In this sense, K_{ATP} channels could act as metabolic gatekeepers of electrical activity, allowing the cells to recover their energy balance by adapting electrical activity of neurones and synapses to their intrinsic energy levels[7].

Initial evidence for the distribution of K_{ATP} channels in the brain came from binding studies using labelled sulfonylureas. Not only was there evidence for K_{ATP} channel expression in pyramidal and other cortical neurones, but the highest density of binding sites was found in the basal ganglia, in particular in the GABA-containing output nuclei: the globus pallidus and the substantia nigra pars reticulata (SNr)[8,9]. The strategic placement of K_{ATP} channels on excitatory or inhibitory neurones could result in the control of network activity, which under conditions of impaired neuronal energy metabolism drives seizure generation and propagation. The GABA-containing neurones in the SNr form a key structure controlling the propagation of seizures[10] because these neurones receive several inputs from basal ganglia nuclei, including a massive GABAergic projection from striatonigral neurones, and in turn project to thalamic targets[11]. Pharmacological inhibition or selective lesion of these SNr neurones reduces the spread of epileptic activity in the brain[10]. Postsynaptic K_{ATP} channels that interfere with somatodendritic action potential generation, and presynaptic K_{ATP} channels that limit neurotransmitter release could contribute to this network control. Indeed, functional and lesion studies indicated that postsynaptic K_{ATP} channels on GABA-containing projection neurones, in addition to presynaptic K_{ATP} channels on GABAergic terminals of striatal projection neurones, contribute to this 'hot spot' of K_{ATP} channel expression in the SNr (Refs 12–14).

Involvement of K_{ATP} channels in seizure propagation

In 1990, Amoroso and colleagues showed that K_{ATP} channel opening reduced GABA release in the SNr and they were the first to speculate that K_{ATP} channels in the substantia nigra might be involved in the control of seizure propagation[15]. An elegant study by Yamada and colleagues now provides direct experimental evidence for the concept of K_{ATP}-channel-mediated seizure control[16]. The research teams of Nobuya Inagaki and Susumu Seino analysed a K_{ATP} channel knockout mouse, where the gene encoding Kir6.2 was inactivated[17], to address the key question of whether K_{ATP} channels are involved in hypoxia-induced seizures. Their behavioural data, combined with electroencephalogram (EEG) and electromyogram (EMG) recordings, clearly demonstrate that K_{ATP} knockout mice possess a dramatically reduced threshold for hypoxia-induced seizure generation. Additional support for a role of neuronal K_{ATP} channels in the dynamic control of the seizure threshold comes from a recent complementary study by Hernandez-Sanchez and co-workers[18]. These authors generated transgenic mice that selectively overexpressed the K_{ATP} channel β-subunit SUR1 in forebrain structures, under the control of a Ca^{2+}–calmodulin kinase promoter, and found that not only was there a significant increase in the threshold of kainate-induced seizures, but also a resistance to excitotoxic neuronal damage in these SUR1-overexpressing animals. These studies examine both ends of a spectrum of K_{ATP} channel expression – complete loss and tenfold overexpression – and provide strong support that the number of active neuronal K_{ATP} channels dynamically controls the seizure threshold in the brain. In this context, it will be relevant to understand regulatory mechanisms such as ischaemic preconditioning that might tune the expression level of neuronal K_{ATP} channels *in vivo* and thus determine the level of protection[19].

Yamada and colleagues[16] also present electrophysiological evidence at the cellular level that postsynaptic K_{ATP} channels alone are sufficient to reduce the activity of the GABA-containing SNr neurones in response to hypoxia. In SNr neurones from knockout mice, the reduction of spontaneous discharge in response to hypoxia is not only completely lost but is converted into a hypoxia-mediated excitation. This indicates that K_{ATP} channel activation is an important counterbalancing mechanism that offsets increased excitability induced by impaired metabolism. *En passant*, their data also demonstrate that the pore-forming subunit Kir6.2 is an essential component of K_{ATP} channels in SNr neurones, consistent with previous biophysical and single-cell reverse-transcriptase polymerase chain reaction (RT-PCR) studies[14,20]. This implies that K_{ATP} channels in the pancreatic β-cell and in metabolically sensitive neurones all share the same molecular makeup of Kir6.2 and SUR1 subunits[4,6,17,20,21]. Consequently, the selective pharmacological targeting of the SNr K_{ATP} channels as suggested by Yamada and co-workers might be difficult. However, it will be important to investigate whether clinically used K_{ATP} channel openers such as diazoxide, which are used to treat hypertension, or K_{ATP} channel blockers such as sulfonylureas, which are used in the treatment of diabetes mellitus type II, also have effects on neuronal K_{ATP} channels in clinically relevant concentrations and thus on ischaemic tolerance and seizure threshold.

Yamada and colleagues convincingly demonstrate a special role of K_{ATP} channels in the SNr as a crucial seizure gate. However, to provide direct proof, a new generation of transgenic mouse models with specific alteration of K_{ATP} channel expression in SNr neurones might be necessary. This important study has provided a large step towards a better understanding of the physiological role of these metabolic gatekeeper channels in the brain.

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A role for neuronal K_{ATP} channels in metabolic control of the seizure gate

Response from Yamada and Inagaki

As noted by Birgit Liss and Jochen Roeper, the subunit combinations of the ATP-sensitive K^+ (K_{ATP}) channels are important in the analysis of the function of these channels in the CNS. Indeed, Roeper's group has shown by a combination of patch-clamp and single-cell reverse-transcriptase polymerase chain reaction (RT-PCR) that there are three populations of dopamine-containing substantia nigra pars compacta (SNc) neurons with different K_{ATP} channel subunit expression profiles and distinct metabolic and drug sensitivities, and that GABA-containing SNc interneurons do not express any of the K_{ATP} channel subunits[1]. This clearly indicates that pharmacological studies should carefully evaluate the functional roles of K_{ATP} channels in brain nuclei comprising heterogeneous neuronal populations that express different subunit combinations. In contrast to the SNc, the substantia nigra pars reticulata (SNr) expresses a homogenous K_{ATP} channel subunit profile with SUR1 and Kir6.2 coexpression in all GABA-containing neurons[1].

The SNr, the major output nucleus of the basal ganglia, is considered to be a key structure that controls the propagation of generalized seizures[2]. A striking feature of the SNr is that almost all of the neurons are spontaneously very active. Their firing rate of up to 100 spikes per second is among the highest in the CNS and is a crucial determinant of basal ganglia functions[3].

Given that spike generation, maintenance of membrane potential, and neurotransmitter synthesis all depend on glucose and oxygen, insufficiency of either glucose or oxygen might lead to an earlier decrease in the cytosolic ATP concentration in this nucleus than in other 'relatively silent' brain areas such as the cerebral cortex and hippocampus. Our data using mice that lack the Kir6.2 subunit of the K_{ATP} channel seemed to support this hypothesis, and we proposed that neurons in the SNr act as a sensor for hypoxia[4].

K_{ATP} channels are expressed in varying abundance in the brain. For example, a recent study found that K_{ATP} channels comprising Kir6.2 and SUR1 were expressed in 58% of hippocampal CA1 interneurons[5]. These channels are also activated by energy depletion[6]. What is the specific role of K_{ATP} channels in the SNr? SNr neurons innervate diverse motor-related target nuclei at long distances, including not only the ventral thalamic nuclei but also the midbrain superior colliculus and the pedunculopontine nucleus in the brain stem[7]. These nuclei are involved in a broad spectrum of motor control[8]. Thus, abrupt silence in the SNr GABA-containing projection neurons in synchrony during the early phase of brain metabolic deficiency might well convey a signal to all these target nuclei as massive disinhibition. Further study is required to clarify the mechanisms of altered function at the target regions in the control of seizure during hypoxia[2].

There is a larger increase in the cerebral metabolic rate of oxygen ($CMRO_2$) during seizure than under any other circumstance[9]. This rapid increase in energy demand associated with the onset of seizure produces a rapid fall in brain energy metabolites. Hence, nigral suppression of seizure propagation might protect from brain damage by reducing the energy consumption rate in the whole brain.

Quantification of the dynamic changes in intracellular ATP concentrations in different cells and regions during normal and pathophysiological conditions is necessary to clarify the various activities of the K_{ATP} channels sufficiently to contemplate

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