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Chapter 2

ACETALDEHYDE AND ION CHANNELS

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ABSTRACT

The relevance of acetaldehyde, the primary metabolite of ethanol oxidation, in the central effects of ethanol has been a conflicting issue. In recent years evidence has accumulated that at least some of the effects on the brain that have been attributed to ethanol result from the action of acetaldehyde. However, basic knowledge about the exact neurochemical mechanisms of acetaldehyde and its molecular targets, such as ion channels, is poor. Ion channels represent integral membrane proteins which play a pivotal role in numerous fundamental physiological processes such as muscle contraction, hormone secretion, or neuronal signaling. Experimental findings suggest that acetaldehyde affects a variety of different types of ion channels and has peripheral as well as central implications. Only a few data provide insight into the response of ion channels under physiological conditions where ethanol and acetaldehyde are simultaneously present. This review gives a resume of the current state of knowledge and highlights the relation between acetaldehyde and ion channels.

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ABBREVIATIONS

ADH	alcohol dehydrogenase
ALDH	aldehyde dehydrogenase
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATP	adenosine triphosphate
BK	big (maxi) calcium-activated potassium channel
Ca^{2+}	calcium ion
$[Ca^{2+}]i$	intracellular free calcium concentration
cAMP	cyclic adenosine monophosphate
CFTR	cystic fibrosis transmembrane conductance regulator
Cl^-	chloride ion
CNMA	trans-cinnamaldehyde
CPP	conditioned place preference
CYP2E1	cytochrome P_{450}2E1
DAT	dopamine transporter
EC_{50}	half maximal effective concentration
ENaC	epithelial sodium channel
$GABA_A$-R	γ-aminobutyric acid A receptor
ρ1 $GABA_C$-R	former name for $GABA_A$ ρ1-R (γ-aminobutyric acid A rho 1 receptor)
GIRK2	G-protein coupled inward rectifying potassium channel 2
GluR1/2/6	glutamate receptor 1/2/6 subunits
H^+	hydrogen ion, proton
HEK293T	human embryonic kidney cells
5-HT_3R	5-hydroxytryptamine- type 3 receptor
I_A	A-type potassium current
I_h	hyperpolarization activated inward current
IP_3R	inositol-1,4,5-triphosphate receptor
K^+	potassium ion
KA2	kainate receptor subunit 2
nAChR	nicotinic acetylcholine receptor
Na^+	sodium ion
Na^+/K^+-ATPase	sodium-potassium pump

NE	norepinephrine
NMDA	N-methyl-D-aspartate
NR1a/2A	N-methyl-D-aspartate receptor 1a/2A subunits
PC-12	pheochromocytoma clonal cells
PGE2	prostaglandins
PKC	protein kinase
pS	pico Siemens
ROS	reactive oxygen species
RyR	ryanodine receptor
SCNN-1	sodium channel non neuronal 1
SR	sarcoplasmic reticulum
STX	saxitoxin
TRP	transient receptor potential channel
TRPA1	transient receptor potential channel A1
TTX	tetrodotoxin
VDAC	voltage dependent anion channels
VGSC	voltage gated sodium channels

INTRODUCTION

Acetaldehyde

Acetaldehyde is the primary product derived from oxidative metabolism of ethanol (alcohol). Albeit ethanol is extensively investigated due to its social relevance, knowledge about acetaldehyde's physiological or patho-physiological effects and its molecular targets is at its infancy. Peripheral accumulation of acetaldehyde in the blood is assumed to account for aversion by producing unpleasant physical symptoms (Eriksson, 2001). On the other hand, in the brain acetaldehyde is supposed to be partially responsible for the rewarding and reinforcing effects of ethanol (Rodd-Henricks et al., 2002; Quertemont et al., 2005a,b; Deng and Deitrich, 2008, Karahanian et al., 2011). Although the role of acetaldehyde in the central effects of ethanol has been a controversial issue new evidence suggests that acetaldehyde is primarily responsible for at least some of the effects on the brain that so far have been attributed to ethanol (Quertemont et al., 2005a,b).

The main enzymes involved in ethanol degradation and hence in acetaldehyde production are the cytosolic alcohol dehydrogenase (ADH), the microsomal cytochrome P_{450}2E1 (CYP2E1) and the peroxisomal catalase

(Lieber, 2004). Aldehyde dehydrogenase (ALDH) which is present in all cellular compartments mediates further transformation of acetaldehyde into the less toxic compound acetate (Deng and Deitrich, 2008). As a water and lipid soluble substance ethanol is able to freely cross cell membranes, to enter cells and to diffuse into all organs including the brain. In contrast, for acetaldehyde it is still in discussion whether effective amounts derived from peripheral ethanol metabolism can permeate the blood brain barrier and reach the brain (Deng and Deitrich, 2008; Correa et al., 2011). This raises the question - how then can acetaldehyde in reasonable amounts appear in the brain? There is now accumulating evidence that ethanol metabolism takes place locally within the brain (Aragon et al., 1992; Gill et al., 1992; Zimatkin and Lindros, 1996). Within the central nervous system the increment of acetaldehyde is predominantly (~60%) mediated by the ethanol oxidative enzyme catalase. In addition to catalase, CYP2E1 (~20%) is involved in central formation of acetaldehyde from ethanol, while ADH appears to play a minor role in this context. Other, still unknown enzymatic pathways may also be involved (Zimatkin et al., 2006; Zimatkin and Buben, 2007).

Given that acetaldehyde is produced within the brain, ethanol and its metabolite may occur simultaneously under physiological conditions after oral alcohol consumption. Different alternative models arise from the concurrent availability of both molecules. The “Ethanol Model” centers ethanol as the acting molecule which provides the pharmacological effects and disregards acetaldehyde as a pure by-product. The converse model is called “Full Prodrug Model”. In this conception acetaldehyde is the key player while ethanol as a simple precursor is supposed to have no effects of its own. In the “Modulation Model” both molecules induce effects and produce the entity of the central effects caused by ethanol consumption in cooperation, modulating each other synergistically as well as antagonistically (Quertemont and Didone, 2006).

Ion Channels

Ion channels are pore-forming transmembranal proteins that are found in all types of cells within the plasma membrane as well as in their organelles. Ion channels constitute hydrophilic pathways for positively charged cations, mainly [sodium (Na^+), potassium (K^+), hydrogen (H^+), calcium (Ca^{2+})] or negatively charged anions [chloride (Cl^-)] across the hydrophobic membrane. Ions can flow passively into as well as out of the cell depending on their electro-chemical gradient without any direct energy consumption. The energy

for ion passage resides in their electro-chemical gradient whose set up is energy dependent (i.e. Na^+/K^+ ATPase or sodium-potassium pump). The flux of ions through the channels is controlled by their "gating" properties. Gating can change the protein conformation rapidly alternating between open and closed states. Gating can be achieved by several factors including membrane potential changes (as in action potentials), binding of ligands, like hormones or neurotransmitters (as in synaptic transmission), by mechanical impact (such as in mechano-receptors, i.e. touch receptors or hair cells) or second messengers like Ca^{2+} or cyclic nucleotides, causing phosphorylation/dephosphorylation processes or temperature alteration. Accordingly ion channels can be classified by their gating factor: voltage-gated, ligand-gated, mechano-sensitive, cyclic nucleotide gated ion channels, or non-gated. A further prominent feature of ion channels is the type of ion which can pass through the pore in the open state (called "selectivity"), since many ion channels are - more or less exclusively - selective for a certain type of ion.

The diversity of ion channels is immense reflecting their particular importance in a multiplicity of physiological processes such as generation and maintenance of the membrane resting potential, generation and propagation of action potentials, signal transduction, muscle contraction, hormone secretion, neurotransmitter release, volume regulation, growth, proliferation or apoptosis. A further feature of many ion channels is their property to pass into an "inactivation" status, where the channel is non-conductive but not in the closed resting state. This is achieved either by a part of the channel protein or an auxiliary protein occluding the pore (Hille, 2001).

Ethanol is considered a centrally active compound to directly act on a number of ion channels and receptors on the molecular level. With regard to possible acetaldehyde actions many typical molecular targets of ethanol have been addressed, since it is of particular interest whether the pharmacological effects of ethanol may be mediated or modulated by acetaldehyde. In this review we attempt to summarize and integrate recent findings on the interaction of ethanol and acetaldehyde on ion channels. We mainly focus on both voltage-gated and ligand-gated ion channels located in the plasma membrane or in cell organelles. Studies dealing with possible acetaldehyde targets are discussed also with regard to missing implications and inconsistencies. Table 1 gives a brief overview of studies on acetaldehyde action and ion channels/receptors.

Table 1. Overview of studies on acetaldehyde effects on ion channels, receptors and currents

Ion channels / Currents	Effect	[ACA]	Cell type	Reference
Sodium				
ENaC (SCNN-1)	▲	178 mM	A6, distal nephron epithelial cell line, *Xenopus laevis*	Bao et al., 2012
VGSC	—	1 & 3 µM	PC-12, rat adrenal medullary tumors	Tsai et al., 2000
	—	75 - 150 mM	rat skeletal muscle cell	Brodie and Sampson, 1990
fast transient inward current	—	0.5 - 1 mM	single atrial myocytes, bullfrog	Chen et al., 2012
Potassium				
BK channels	▼	<100 µM + 30 mM EtOH	GH3, rat pituitary tumor cells	Handlechner et al., 2013
outward delayed rectifier K^+	—	0.5 - 1 mM	single atrial myocytes, bullfrog	Chen et al., 2012
inward rectifying K^+	—			
I_A currents	▼	<1 µM	dopaminergic neurons, rat brain, ventral tegmental area	Melis et al., 2007
I_h currents	▲			
GIRK2	—	1, 100 & 1000 µM	heterologous expression in *Xenopus laevis* oocytes	Mascia et al., 2001
Calcium				
Ca^{2+} transient	▲ ▼	1-10 µM 100 µM	rat ventricular myocytes	Oba et al., 2008
	▲ ▼	< 3 mM > 3mM	rat myocardial tissue	Brown et al., 1999
L-type Ca^{2+} channels	▲	300 - 500 µM	single atrial myocytes, bullfrog	Chen et al., 2012
	▼	10 - 30 mM	vascular smooth muscle cells, rat thoracic aorta	Morales et al., 1996
	▼	250 mg/kg	neuronal tissue, cerebral cortex, rat brain	Bergamaschi et al., 1988

Ion channels / Currents	Effect	[ACA]	Cell type	Reference
RyR	▲	1 - 100 µM	rabbit RyR2 in lipid bilayer	Oba et al., 2008
	▲	50 - 200 µM	frog RyR and rabbit RyR1, in lipid bilayer	Oba and Maeno, 2004
	▼	1 mM		
IP_3R	▲	75 - 200 µM	brain microvascular endothelial cells, human temporal cortex	Haorah et al., 2007
TRPA1	▲	<1 mM	heterologous expression in HEK293T, mouse trigeminal neurons	Bang et al., 2007
Chloride (Anions)				
CFTR	▬	1 & 5 mM	pancreatic ductal epithelial cells, guinea pig	Judák et al., 2014
VDAC	▼	100 - 500 µM	rat hepatocytes	Holmuhamedov et al., 2012
alpha-1-glycine receptor	▲	1 & 10 µM	heterologous expression in *Xenopus laevis* oocytes	Mascia et al., 2001
$GABA_A$–R	▬	1, 100 & 1000 µM (+/- EtOH)		
ρ1 $GABA_C$–R	▬			
Ligand-gated (non selective) cation channels				
5-HT_{3A}R	▬	10 - 300 µM (+/- EtOH)	heterologous expression in *Xenopus laevis* oocytes	Mascia et al., 2001
NR1a/NR2A NMDA	▬	10 & 100 µM (+/-EtOH)		
GluR1/GluR2 AMPA	▬			
GluR6/KA2 kainate	▬			
nAChR	▬			

(ACA - acetaldehyde, EtOH - ethanol; ▲ - increment, ▼ - decrement, ▬ - no effect).

Voltage-Gated Ion Channels

Voltage-dependent ion channels are activated by membrane depolarization or hyperpolarization. Basically, many voltage-dependent ion channel proteins consist of four homologous domains (Na^+ and Ca^{2+} channels) or four separate identical α-subunits (K^+ channels), each consisting of six transmembranal segments (S1-S6), an extracellular poor-loop between S5 and S6 and a voltage-sensor including segments S1-S4 (with a focus on S4). Since electrical signaling is important for the flow of information predominantly among excitable cells, voltage-gated ion channels are essentially involved in a great variety of physiological processes, from muscle contraction, secretion to signal processing in the brain (for review see Yu et al., 2005; Dai et al., 2009).

Voltage-Gated Sodium Channels

The family of voltage gated Na^+ channels comprises nine members, $Na_v1.1$-$Na_v1.9$, where most are sensitive to tetrodotoxin (TTX, a puffer fish toxin that specifically blocks these Na^+ channels) whereas some other Na^+ channels which bear minute aberrations in their amino acid texture are TTX resistant. Voltage-gated sodium channels are responsible for the generation and propagation of action potentials. If membrane depolarization exceeds a certain threshold, voltage-dependent Na^+ channels open and allow for Na^+ influx along their electro-chemical gradient to initiate the rising phase of action potentials. Na^+ currents are terminated by a fast inherent closure of the channels (called "inactivation") which is involved in the repolarization of action potentials. The delayed opening of voltage-dependent K^+ channels drives the membrane potential towards the K^+ equilibrium potential speeding up repolarization of the action potential and finally restoration of the membrane resting potential (Catterall, 2012).

Frequency and rate of rise of action potentials correlate with the open probability and density of Na^+ channels (Brodie et al, 1989). Acute ethanol has been shown to block electrical activity by depressing Na^+ influx in isolated rat brain synaptosomes (Harris and Bruno, 1985a,b; Mullin and Hunt, 1985) without changing the quantity of Na^+ channels. Measurements using the specific binding of [^{3}H] saxitoxin (STX, another Na^+ channel specific blocker) were used to assess the number of Na^+ channels (Mullin and Hunt, 1987). As shown previously for Ca^{2+} (Messing et al., 1986), Brodie and Sampson (1990) found that acute ethanol depresses voltage-gated Na^+ uptake which is

compensated during chronic ethanol by an increase of Na^+ uptake caused by an increase of Na^+ channel density. In cultured rat muscle cells extremely high acute ethanol (150 mM) was shown to account for omission of spontaneous activity within 5 minutes (Brodie and Sampson, 1987a,b), and a gradual depolarization of the membrane resting potential (~13 mV) (Brodie and Sampson, 1990). Application of external acetaldehyde (75 - 150 mM) on myotubes had no impact on either membrane resting potential properties or spontaneous activity and therefore was not considered being involved in ethanol-induced changes in action potential frequency and appearance. Investigation of a possible influence of acetaldehyde on the ethanol-mediated compensatory increase in Na^+ channel density was not addressed in this study (Brodie and Sampson, 1990).

A further study by Tsai et al. (2000) supported the finding that acetaldehyde had no impact on STX-sensitive Na^+ channels when applied to the external face of the membrane. Trans-cinnamaldehyde (CNMA) was used as a norepinephrine (NE) releasing drug in pheochromocytoma clonal (PC-12) cells (which are derived from rat adrenal medullary tumors). This Ca^{2+} and cAMP (cyclic adenosine monophosphate) dependent NE release by CNMA primarily relied on membrane depolarization (Green and Rein, 1977; Cheng et al., 2000). While CNMA was able to trigger the NE release activation mechanisms, in contrast incubation with external acetaldehyde (1 and 3 μM) did not provoke this effect. STX decreased the CNMA-induced depolarization, indicating that voltage-gated Na^+ channels actually take part in this mechanism, however, they were not affected by external acetaldehyde (Tsai et al., 2000).

Voltage-Gated Potassium Channels

Potassium ion channels exhibit the largest and most prevalent type of ion channels in excitable and non-excitable cells either expressed as voltage-activated or non voltage-activated variety. Within the class of potassium channels voltage-dependent types form the largest family grouped into 12 subfamilies (Wulff et al., 2009).

A-Type Potassium Current (I_A) and Hyperpolarization-Activated Inward Current (I_h)

The A-type potassium channel is a transient outward-rectifier with fast inactivation kinetics (Antz and Fakler, 1998). In these channels an inactivation particle ("ball-and-chain"-mechanism: ball-shaped amino acid configuration on a string) rapidly closes the pore although the activation gate is still open. Consequently, the channel can appear in three different states: in an activatable state where the channel is closed, in the open state where it is conducting ions, and in an inactivated state where the "ball" plugging the channel prevents ion conduction (Armstrong and Bezanilla, 1977; Armstrong, 1981; Miller, 1991). The rapid inactivation process is essential for the regulation of the firing rate of excitable cells, since excitability between action potentials depends on modification of I_A (Antz and Fakler, 1998; Hille, 2001).

The hyperpolarization-activated inward current is also called "anomalous-rectifier current" since activation by negative potentials is rather unusual among voltage-gated ion channels. I_h (or HCN, hyperpolarization-activated cyclic nucleotide-gated cation channel) channels are non-selective cation channels with a 3-5 fold higher permeability to K^+ than to Na^+. However, due to their activation at potentials near to the K^+ equilibrium potential I_h currents are predominantly carried by Na^+. The inwardly directed flux of Na^+ depolarizes the membrane and facilitates action potential firing of heart and nerve cells. Hence the channels are also called “pacemaker channels” referring to their role in controlling rhythmic activity of cardiac pacemaker cells and the generation of spontaneous firing in neurons (DiFrancesco, 1993; Pape, 1996; for review see Biel et al. 2009).

The positive motivational properties of ethanol are considered to rely on the activation of the mesolimbic dopaminergic system. Even though peripheral accumulation of acetaldehyde produces aversion, evidence suggests that its central availability may have a mediating role in this context. Melis et al. (2007) confirmed the assumption that acetaldehyde is absolutely required for the ethanol induced enhancement of dopaminergic transmission in the ventral tegmental area (VTA) in the rat brain. In electrophysiological experiments two ionic currents were found to account for the increase in dopaminergic neuronal activity – namely, both I_A and I_h. I_A currents affect repolarization and hence control action potential spacing and frequency (Grace 1991). Acetaldehyde (1 µM) applied externally was shown to inhibit I_A currents, thereby enhancing dopaminergic neuronal firing frequency. Pretreatment with the catalase blocker, 3-aminotriazole (3-AT), which prevents acetaldehyde formation from

ethanol within the cell, suppressed the ethanol effect of firing enhancement and the inhibition of I_A currents (Melis et al., 2007). In contrast to I_A, I_h currents reduce after-hyperpolarization intervals and promote excitability and spike frequency (Neuhoff et al., 2002). Application of external acetaldehyde (1 µM) amplified the effect of I_h currents. In summary, reduction of I_A and activation of I_h augment dopamine induced neuronal firing in the VTA in response to acetaldehyde. The *in vitro* findings were supported by conditioned place preference (CPP) experiments, showing that ethanol-induced CPP requires acetaldehyde. Additional analyses by microdialysis demonstrate that, dopamine release was increased by ethanol only in the presence of acetaldehyde (Melis et al., 2007).

Maxi Calcium-Activated Potassium Channels (BK)

BK is the abbreviation for "Big K^+ ", since a large unitary ion conductance of 100-300 pico Siemens (pS) is a key feature of these channels. Regulation of BK channel activity is initiated by two pivotal factors, by membrane depolarization and by an increase of free intracellular calcium concentration ($[Ca^{2+}]i$), which enables this channel to act as a molecular integrator comprising the intracellular Ca^{2+} messenger system and the electrical state of the cell membrane. BK channels exhibit a great variety of functions like action potential repolarization, regulation of blood pressure, hormones secretion or transmitter release (Toro et al., 1998; Weiger et al., 2002; Hermann et al., 2012). BK channel activity can be modulated by a plethora of factors, including changes in pH, redox potential, protein kinases/phosphatases (for reviews see Weiger at al., 2002; Hou et al., 2009; Kyle and Braun, 2014), or by interactions with auxiliary β- (Weiger et al., 2000; Torres et al., 2007) or γ-subunits (Yan and Aldrich, 2012). BK channels are also a prominent cellular target for ethanol which is well known to enhance BK channel activity in a dose dependent manner, probably via a Ca^{2+} and protein kinase C (PKC) dependent mechanism (Dopico et al., 1996; Jakab et al., 1997; Dopico et al., 1999; Brodie et al., 2007). The ethanol-mediated activation of BK channels disposes the cell to reduce hormone secretion and transmitter release through hyperpolarization of the membrane potential (Dopico et al., 1999). Ethanol modulation of BK channels influences neuronal excitability, cerebrovascular tone, brain function and finally behavior (Davies et al., 2003; Brodie et al., 2007; Liu et al., 2008).

In a recent study of our lab application of ethanol and acetaldehyde was used separately, simultaneously, or successively in order to investigate their individual effects as well as their interactions (Handlechner et al., 2013). Patch Clamp techniques were employed using single channel recordings of excised, cell-free patches which allow for the study of channel properties without interference of other cellular influences. In short-term experiments extracellular application of 30 mM ethanol (about 1.3‰) enhanced BK channel open probability within seconds (Jakab et al., 1997). The ethanol-mediated increase in activity was completely abolished by intracellular acetaldehyde in a concentration- and Ca^{2+} dependent manner. The effective concentration of acetaldehyde which blocked the half maximal effect of ethanol (EC_{50}) was approximately 400 nM. The countervailing effect of acetaldehyde was found to depend on the succession of application. BK activation by ethanol was not inducible subsequent to a pre-exposure to acetaldehyde. Moreover, the acetaldehyde counteraction of the ethanol-mediated enhancement of BK channel activity was a lasting effect which persisted after acetaldehyde removal, a situation which could prevail in chronic alcohol abuse. BK single channel conductance was not affected but mean channel open time was reduced significantly which points to an interaction of acetaldehyde with the channel gating process. Whereas intracellular acetaldehyde reduced mean channel open time and countervailed BK activation by ethanol, extracellular applied acetaldehyde (0.1 – 10 mM) failed to affect BK channels. We interpret this as indication for acetaldehyde not being able to cross the cell membrane in the short time range (minutes) used in our experiments. Hence acetaldehyde appears to specifically target the channel protein from the internal side, probably close to or at the ethanol binding site (Bukiya et al., 2014). Furthermore, the modulation of ethanol activation through acetaldehyde appears to reflect a specific interaction with BK channels, since acetaldehyde did not impede hypotonicity-induced BK activation (Handlechner et al., 2013). Additionally, long-term analyses over a period of 15 minutes showed that the initial increase in BK channel open probability provoked by ethanol is transient and recovers to control levels within minutes. Whereas acetaldehyde applied by its own did not lead to an appreciable modification of BK channel activity, the continuous presence of acetaldehyde and ethanol in combination decreased BK channel activity significantly in the long run. This decline of BK channel activity even well below the pre-application level may indicate a second mechanism of action for acetaldehyde when applied together with ethanol or might indicate that

priming of the channels by ethanol is necessary for the acetaldehyde effect to take place (Handlechner et al. 2014).

Under physiologically relevant conditions acetaldehyde and ethanol are present simultaneously in the brain after drinking. Despite this fact, usually each of these chemicals is investigated separately. Our findings based on a different experimental design suggest a mutual interference of ethanol and acetaldehyde with BK channels, ascribing acetaldehyde a decisive role in the context of ethanol action and in ethanol tolerance (Handlechner et al., 2013).

Calcium and Voltage-Gated Calcium Channels

Ca^{2+} is an important second messenger governing intracellular signal transduction in all types of cells. Compared to extracellular concentrations $[Ca^{2+}]i$ levels are 10.000 fold lower. Abnormally increased intracellular Ca^{2+} levels are associated with excitotoxicity and may result in cell death. Hence, maintenance of Ca^{2+} homeostasis is pivotal for cell function. $[Ca^{2+}]i$ can be altered by Ca^{2+} influx from the extracellular space or by Ca^{2+} release from intracellular Ca^{2+} stores (Yamakage and Namiki, 2002; Catterall, 2011). Ca^{2+} influx is essentially involved in processes including contraction, secretion, neurotransmission or gene expression (Yu and Catterall, 2004). Two classes of Ca^{2+} channels are distinguished due to their voltage-dependence: the low-voltage-activated T-type Ca^{2+} channel and the high-voltage activated group with N-, P/Q-, and L-type Ca^{2+} channels (for reviews see Yamakage and Namiki, 2002; Catterall, 2011).

L-Type Calcium Channels

High-voltage-activated L (long-lasting openings) -type Ca^{2+} channels are widely distributed in skeletal muscle cells, in cardiac and smooth muscle myocytes, in endocrine cells, and in neuronal cell bodies. The group consists of 4 different types, all of which are blocked by dihydropyridine. They provide numerous functions including excitation-contraction-coupling, cardiac pacemaking, hormone secretion, and regulation of transcription or synaptic integration (Catterall et al., 2005, 2011).

Acute ethanol was shown to reduce voltage-dependent Ca^{2+} uptake (Harris and Hood, 1980). This can lead to a compensatory increment in Ca^{2+} uptake in chronic ethanol due to an increase in Ca^{2+} channel density in clonal

neural cells (Messing et al., 1986). The findings were confirmed by measuring the binding of ^{3}H-nitrendipine (^{3}H-NTP, a Ca^{2+} antagonist) (Bergamaschi et al., 1988). In addition Bergamaschi et al. (1988) showed that intraperitoneal injection of acetaldehyde (250 mg/kg body weight) produces similar effects on L-type Ca^{2+} channels in the rat brain. The enhanced density of active Ca^{2+} channels is suggested to represent an adaptive neuronal response to ethanol. This adaptation enables the regeneration of an adequate Ca^{2+} influx as it disposes the cell to counteract the inhibitory effect of acute ethanol on L-type Ca^{2+} channels (Harris and Hood, 1980). Hence, acetaldehyde, which displayed a faster onset of action compared to ethanol, may be involved in ethanol action on neuronal L-type Ca^{2+} channels with a distinct impact on neuronal excitability (Bergamaschi et al., 1988).

Externally applied acetaldehyde (10 and 30 mM) also inhibits L-type Ca^{2+} channels in aorta vascular smooth muscle cells, preventing K^{+} elicited contraction (Morales et al., 1997). Vascular smooth muscle contraction by depolarization of the cell membrane is impaired by acetaldehyde which acts as an acute vasorelaxant. Since contraction needs elevation of $[Ca^{2+}]i$, operated by L-type Ca^{2+} channels in the sarcolemma and ryanodine receptor (RyR) mediated Ca^{2+} efflux from the sarcoplasmic reticulum (SR), the loss of contractility caused by acetaldehyde is probably based on changes in the action and number of voltage gated Ca^{2+} channels. The influence of acetaldehyde on vascular membrane ionic currents indicates a possible role in the development of hypertension observed during chronic alcohol abuse. Using the whole-cell patch clamp technique the effect of 10 and 30 mM acetaldehyde on the magnitude and voltage dependence of inward Ca^{2+} currents was examined. A significant decline of Ca^{2+} inward current was observed at 30 mM acetaldehyde within 4 minutes with a further decrement within 8 minutes. At 10 mM the decline was delayed in time. Although the concentrations used in this study were very high and not relevant to normal physiological conditions, the observed progressive inhibitory action at the lower acetaldehyde concentration of 10 mM over a long time period may indicate an involvement in excessive long-term alcohol consumption (Morales et al., 1997).

The contractile response to NE or K^{+} was diminished by acute external acetaldehyde (30 mM) in rat aorta smooth muscle cells, most likely caused by an acetaldehyde-induced inhibition of Ca^{2+} influx from extracellular space (Brown and Savage, 1996). In corpus cavernosum smooth muscles of rabbits externally applied acetaldehyde was shown to reduce contractility - and in part relaxation - already at a concentration of 100 µM (Kim et al., 2000). In another

study, however, externally applied acetaldehyde at similar low concentrations was shown to enhance spontaneous phasic contractile activity in vascular smooth muscle cells of the rat aorta (Altura et al., 1978).

The damaging impact of acute and chronic ethanol on cardiac muscle induces arrhythmias and ventricular dysfunction. Chen et al. (2012) investigated the action of externally applied acetaldehyde on Ca^{2+} currents in single atrial bullfrog myocytes. In contrast to vascular smooth muscle cells and neuronal cells as described above acetaldehyde increased L-type Ca^{2+} channels in myocytes with an EC_{50} of 300 µM. Likely due to the increment of Ca^{2+} influx the contractile force was enhanced in a dose-dependent manner with an EC_{50} of 500 µM. Furthermore, action potential overshoot and plateau phase were augmented by 300 µM and 500 µM acetaldehyde, respectively. Fast transient inward Na^{+} currents were not affected by acetaldehyde and, hence, were not considered to contribute to the enhanced overshoot during the depolarization phase. The duration of action potentials was altered in as much as the time interval needed for a 90% repolarization was shortened. The rate of Ca^{2+} current inactivation and the rate of K^{+} current activation are the major determinants for the duration of the action potential repolarization (Rasmusson et al., 1990; Ono and Giles, 1991). Since the outward-delayed rectifier K^{+} current and the inward rectifying K^{+} current are not changed in response to acetaldehyde the shortening of action potentials may result from an acetaldehyde mediated acceleration of the current decay during Ca^{2+} current inactivation (Chen et al., 2012).

In rat myocardial tissue 1 - 30 mM external acetaldehyde depressed myocardial contraction and myocyte shortening (Ren et al., 1997; Brown et al. 1999). Measurements of intracellular Ca^{2+} transients by means of fura-2 fluorescence revealed a biphasic response: enhancement of internal Ca^{2+} at concentrations below 3 mM, but inhibition at concentrations in excess of 3 mM acetaldehyde. Still, it has to be mentioned that these are unphysiological high concentrations of acetaldehyde. Brown et al. (1999) assumed that the enhancement of the intracellular Ca^{2+} transient at the lower acetaldehyde doses (< 3 mM) is related to beta-adrenergic stimulation of L-type Ca^{2+} channels, while the inhibition by using high doses (> 3 mM) is based on the reduction in either Ca^{2+} entry from the extracellular space or Ca^{2+} release from intracellular stores, or possibly both. Since the intracellular Ca^{2+} transient consists of two components, Ca^{2+} influx through L-type Ca^{2+} channels and the Ca^{2+} efflux from intracellular stores, the observed inhibition can, at least in part, be attributed to an acetaldehyde impact on RyRs, as described in the next section.

Intracellular Calcium Channels

Ryanodine receptors (RyR) are localized predominantly in the SR of skeletal muscle cells (RyR1) and cardiac muscle cells (RyR2). RyR3 is supposed to be the most prominent neuronal isoform, however, simply because it has been originally identified in the brain. RyR3 is distributed in all tissues, and all three isoforms are found in the endoplasmic reticulum (ER) of neuronal cells. RyR is generally responsible for Ca^{2+} release from intracellular stores. In the skeletal muscle RyR is the link between cell membrane depolarization and contraction, known as excitation-contraction coupling. Activation of voltage-dependent L-type Ca^{2+} channels in the plasma membrane leads to Ca^{2+} influx which mediates, by direct or indirect interaction with RyR, further Ca^{2+} release from the SR. In cardiac muscle Ca^{2+} influx from extracellular space mediates Ca^{2+} induced Ca^{2+} release (CICR). Subsequently, the resulting Ca^{2+} transient is sufficient to trigger contraction (Zucchi and Ronca-Testoni 1997; Fill and Copello, 2002).

In isolated rat myocytes the Ca^{2+} transient measured by confocal calcium imaging was enhanced at low concentrations of external acetaldehyde (1 -10 µM), but significantly reduced at 100 µM. Against expectation, single rabbit RyR2, incorporated into a planar lipid bilayer for single channel recordings, showed an increased channel activity in response to acetaldehyde applied to the cytosolic side of the channel in the concentration range from 1 to 100 µM. The Ca^{2+} increment appeared to depend on the cytoplasmic redox potential, since acetaldehyde is a potent RyR2 activator when the redox potential is kept near the resting state. Under reducing conditions or under the influence of oxidative stress RyR2 is hardly activatable by acetaldehyde. The production of reactive oxygen species (ROS) mediated by acetaldehyde alters the intracellular redox potential from a reduced to an oxidized state. Thus, the redox potential appears to convey a protective mechanism that prevents acetaldehyde from disrupting Ca^{2+} handling or disturbing cardiac excitation-contraction coupling (Oba et al., 2008).

Ethanol can induce skeletal muscle dysfunctions, i.e. muscle weakness. Khan (1981) observed a reversible ethanol-mediated suppression of the isometric twitch and tetanic tension in frog muscle fibers. The maximum rate of force was reduced. Externally applied acetaldehyde in higher concentrations (18 mM) produced similar effects, but twitch potentiation was found even at lower doses (<1.8 mM), probably due to an augmentation of Ca^{2+} release from intracellular stores. Inconsistent with these findings, single rabbit RyR1 was shown to be inhibited by cytosolic acetaldehyde at concentrations higher than

1 mM (Oba et al., 2000). However, a further study by Oba and Maeno (2004) revealed that exposure of RyR1 channels to cytosolic acetaldehyde at low doses (50 - 200 µM) enhanced channel activity and intracellular Ca^{2+} release in a dose- and time dependent manner, leading to potentiated twitch tension. Clinical relevant concentrations of acetaldehyde (< 30 µM) did not affect RyR channel activity in skeletal muscle cells which suggests that acetaldehyde does not play a role in acute skeletal muscle dysfunction (Oba and Maeno, 2004).

The inositol-1,4,5-triphosphate receptor (IP_3R) is a ligand gated Ca^{2+} channel usually located in the ER from where it mediates Ca^{2+} release (Berridge, 1993). Exposure (2 h) of primary human brain microvascular endothelial cells (BMVEC) to either ethanol (25 - 100 mM) or acetaldehyde (75 - 200 µM) increased IP_3R protein expression and activity resulting in an augmented intracellular Ca^{2+} release. Since the ethanol-mediated increase was prevented by the ethanol metabolism blocker 4-methylpyrazole (4-MP,) stimulation of intracellular Ca^{2+} release appears to rely on acetaldehyde (Haorah et al., 2007). Acetaldehyde/ROS enhanced IP_3R-gated Ca^{2+} release causes activation of myosin light chain kinase (MLCK) which accounts for intensified phosphorylation of cytoskeletal or tight junction proteins (Brown and Davies, 2002). This phosphorylation processes impair cytoskeleton arrangement and regular tight junction assembly, destroying blood-brain barrier integrity. Blood brain barrier dysfunction is linked to several neurological disorders as well as to neuronal degeneration in chronic alcohol conditions (Haorah et al., 2007).

Voltage-Dependent and Independent Anion Channels

Cl^- channels in general are a heterogeneous group of proteins containing 13 members which differ in molecular structure and physiological function. Cl^- channels are localized in the plasma membrane as well as in the membranes of intracellular organelles. The number of the transmembrane segments can vary from 1 to 12 and the conductance is also very different (from 1 to 200 pS) among the individual representatives. They are involved in ligand-gated postsynaptic transmission, stabilization of the resting membrane potential in muscle cells, depolarization of smooth-muscle, regulation of the cell volume, fluid transport in epithelia and neutralization of H^+ ions in lysosomal vesicles (for reviews see Jentsch et al., 2002; Suzuki et al., 2006).

The voltage dependent anion channel (VDAC), a Cl^--selective porin, is predominantly localized in the outer mitochondrial membrane, although it is

also found in the cell membrane (Jentsch et al., 2002). The VDAC is responsible for the entry and exit of hydrophilic metabolites involved in oxidative phosphorylation through the outer mitochondrial membrane. Hence, all water-soluble metabolites have to pass through a VDAC (Colombini, 2004; Lemasters and Holmuhamedov, 2006). In cultured rat hepatocytes ethanol was shown to reduce permeability to hydrophilic metabolites by inhibiting the VDAC (Holmuhamedov and Lemasters, 2009).

Ureagenesis is a complex metabolic process composed of five biochemical reactions all of which require extensive persistent exchange of metabolites between cytosol and mitochondria. The continuing exchange is impaired when VDACs are inhibited by ethanol. Hence, measurements of ureagenic respiration allows for indirect investigation of the VDAC gating behavior. Ethanol in concentrations from 10 to 200 mM suppressed ureagenic respiration and urea formation, previously increased by application of ureagenic substrates. Ethanol-mediated suppression of ureagenic respiration was partially reversed by inhibitors of the ethanol metabolizing enzymes ADH, CYP2E1 and catalase. These inhibitors prevent acetaldehyde formation. In contrast, inhibition of ALDH leading to accumulation of acetaldehyde enhanced suppression of ureagenic respiration. Furthermore, acetaldehyde added to cultured hepatocytes reduced ureagenic respiration significantly with an IC_{50} (half maximal inhibitory concentration) of 125 µM, and inhibited entry of a 3 kDa rhodamine-conjugated dextran through the outer mitochondrial membrane. Both actions indicate VDAC closure. In summary, acetaldehyde provokes VDAC closure and, hence, mediates suppression of ureagenic respiration in hepatocytes (Holmuhamedov et al., 2012).

The cystic fibrosis transmembrane conductance regulator (CFTR) – a
is not voltage-dependent but cAMP activated and ATP (adenosine triphosphate) gated. CFTR is located exclusively in apical epithelial cell membranes of intestine, airways or secretory glands where it is responsible for transepithelial transport processes, such as regulation of ductal electrolyte and fluid secretion (Jentsch et al., 2002). Functional inhibition of this Cl^- channel may be involved in the pathogenesis of alcoholic pancreatitis. In pancreatic ductal epithelial cells ethanol (10 and 100 mM) augmented basal CFTR currents, but reversibly blocked CFTR currents stimulated by forskolin, a known cAMP - agonist. Both the stimulatory and the inhibitory effect of ethanol were not mimicked by exposure to extracellular acetaldehyde in concentrations of 1 and 5 mM, respectively. This result suggests that acetaldehyde is not involved in pancreatitis inducing processes mediated by CFTR (Judák et al., 2014).

Ligand-Gated Ion Channels

Ligand-gated ion channels change conformation in response to the binding of an adequate ligand (transmitter, hormone, etc.) leading to channel gating and regulation of ion transport through the channel pore. Some channels are selective for cations, others for anions. Ligand-gated ion channels are classified into four superfamilies: members of the cys-loop superfamily are $GABA_A$ and $GABA_C$ (γ-aminobutyric acid) receptors as well as glycine receptors, both of which represent anion channels. Cation channels belonging to this receptor superfamily are nACh (nicotinic acetylcholine) and 5-HT_3 (5-hydroxytryptamine, type 3) receptors. All members of this group contain a characteristic loop formed by a disulfide bond between two cysteine residues. Another superfamily is called glutamate receptors, since all of them are sensitive to glutamate binding. NMDA (N-methyl-D-aspartate), AMPA (*α*-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and kainate receptors are the primary representatives of this superfamily. The TRP (transient receptor potential) channels and the ATP-gated channels are each divided into two more superfamilies. The major task of neuronal ligand-gated ion channels is the translation of a presynaptic chemical information (i.e. neurotransmitter, ATP) into a rapid electrical signal at the postsynaptic cell. Dependent on the type of ion current, activation of a ligand-gated receptor can result in inhibitory or excitatory transmission (Connolly and Wafford, 2004).

Ligand-gated ion channels are major targets of ethanol. Mascia et al. (2001) attempted to explore whether ethanol actions on ligand-gated ion channels may be mediated by acetaldehyde. Ten different representatives of this group were expressed in *Xenopus laevis* oocytes and investigated using the two-electrode voltage clamp technique. Possible effects on the corresponding currents were measured and tested against the currents induced by EC_{5-50} of the particular agonists according to channel type. Three glutamate receptor subtypes (NR1a/NR2A NMDA, GluR1/GluR2 AMPA, GluR6/KA2 kainate) were exposed to external acetaldehyde in concentrations of 10 µM and 100 µM, respectively. None of the glutamate receptor subtypes responded to acetaldehyde exposure, although, each was inhibited by ethanol (Dildy-Mayfield and Harris, 1995; Mirshahi et al., 1998). The same test procedure applied to four members of the cys-loop superfamily yielded similar results. The non-selective ligand-gated cation channel subtypes of nACh ($\alpha_4\beta_2$, $\alpha_4\beta_4$, $\alpha_2\beta_4$), exposed to 10 and 100 µM of external acetaldehyde, did not exhibit any response. The same result was shown for the 5-HT_{3A} -receptors, exposed to 10 - 300 µM of acetaldehyde. Potentiation of function by acute ethanol was

reported for both nACh (Cardoso et al., 1999) and 5-HT_{3A} (Machu and Harris, 1994) receptor currents.

Likewise, neither the Cl^- channel subtype $\alpha_1\beta_2\gamma_{2s}$ $GABA_A$ nor ρ_1 $GABA_C$ was influenced by external acetaldehyde (1, 100, and 1000 µM). $GABA_A$ receptor mediated currents are enhanced by ethanol (Mihic et al., 1994), whereas ρ_1 $GABA_C$ mediated currents are inhibited by low concentrations of ethanol (Mihic and Harris, 1996). The homomeric α_1 glycine receptor was the only member of the ligand-gated ion channel family investigated in this study which was significantly and reversibly enhanced by relatively low (1 µM and 10 µM), but not by high acetaldehyde concentrations (100 and 1000 µM) when applied from the extracellular face of the cell membrane.

Since α_1 glycine receptor currents also increase in response to moderate ethanol concentrations (10 - 200 mM) (Mascia et al., 1996), acetaldehyde might as well act on these receptors, possibly playing a role in the anesthetic action of ethanol. All receptors that were shown to be insensitive to acetaldehyde in this study were further tested for their previously referred responsiveness to ethanol (100 - 200 mM). Additionally, ethanol (100 - 200 mM) and acetaldehyde (10 µM or 100 µM), when applied simultaneously to the external side of the cell membrane, did not interfere with each other which means that acetaldehyde did not affect ethanol action on ligand-gated ion channel currents (Mascia et al., 2001).

Finally, besides the ligand-gated ion channels Mascia et al. (2001) also included two further supposable targets of acetaldehyde into their investigation. Currents of GIRK2, a G-protein coupled inward rectifying K^+ channel, were augmented by 100 mM ethanol (Lewohl et al., 1999) but not affected by external acetaldehyde at concentrations of 10, 100, or 1000 µM. The dopamine transporter (DAT) activity was raised by acute as well as chronic ethanol (Mayfield et al., 2001). Dopamine uptake was measured in DAT expressing oocytes after exposure to external acetaldehyde (10 and 100 µM) for 10 minutes representing the acute situation. Acetaldehyde exposure for 1 and 4 hr corresponds to chronic conditions. Both acute and chronic acetaldehyde treatments did not entail any increment in dopamine uptake revealing no potentiation of the DAT (Mascia et al., 2001).

Epithelial Sodium Channels (ENaC)

The ENaC is also referred to as SCNN-1 (sodium channel non-neuronal-1). It is a constitutively active, amiloride sensitive channel, permeable to

lithium (Li^+), H^+, and Na^+. Due to its similarity to the ENaC/degenerin family and to the acid sensing ion channel (ASIC) ENaC is supposed to be a ligand-gated ion channel (Horisberger and Chraïbi, 2004). The ENaC is located in the apical membrane of polarized epithelial cells where it allows for the reabsorption of Na^+ from the luminal side of the epithelium and Na^+ entry into the cell. On the basolateral side of the membrane a Na^+/K^+-ATPase is responsible for the Na^+ discharge from the cell. K^+ ions leave the epithelial cell through apically situated K^+ channels. ENaC along with Na^+/K^+-ATPase perform an active transepithelial Na^+ transport which ensures salt and water homeostasis in the blood. The maintenance of the composition and volume of the fluids on either side of the epithelium is of pivotal importance in organs with excretory functions including kidney, lung, gastrointestinal tract, sweat and salivary glands. Salt taste perception is also mediated by ENaC located in taste receptor cells (Kellenberger and Schild, 2002). Four homologous subunits (α, β, γ, and δ) can form a functional ENaC, and all of the four subunits are also found in the peripheral and central nervous system. Admittedly, almost nothing is known about their role in the brain, although, an involvement in degenerative disorders and the central control of blood pressure is likely (Giraldez et al. 2013).

In response to acute ethanol excretion of Na^+ was shown to be reduced in rats (Assadi 1989). Based on this changing of electrolyte transport it was suggested that ethanol has an impact on Na^+ transporting cells. Since ethanol metabolism produces ROS like superoxide or hydrogen peroxide (Bailey and Cunninham, 1998; Heaton et al, 2002), evidence pointed to a ROS-mediated effect on ENaC. Ethanol was shown to significantly elevate ENaC activity in distal nephron cells and this effect was mimicked by external acetaldehyde (1% or 178 mM) (Bao et al., 2012). Ethanol generating ROS triggers a signaling cascade leading to enhanced PIP3 (phosphatidyinositol-3-phosphate) interaction with ENaC (Helms et al., 2005; Ma et al., 2007). Due to this interaction both activity and density of ENaC channels in the apical membrane were increased. Whereas ethanol metabolism produces ROS, acetaldehyde enforces the intracellular accumulation of ROS. Acetaldehyde mediates inhibition of SOD1 (superoxide dismutase) (Eom et al., 2007) and catalase (Venkatesan et al., 2007) preventing ROS breakdown. Bao et al. (2012) infer from their experiments that ethanol stimulates ENaC by elevating intracellular ROS probably via acetaldehyde.

Transient Receptor Potential Channels

TRP channels are non-selective, ligand-gated cation channels. Voltage-sensitivity has been reported for many TRPs, but is low compared to classical voltage-dependent ion channels (Zheng, 2013). Almost 30 members of mammalian TRP channel family have been characterized to date. Most of them are located in the cell membrane, some are also found in intracellular organelles (Montell, 2005). Some of the TRP channels are qualified as thermo-sensitive but they are also activated by natural compounds including capsaicin, present in hot chilli peppers, allicin from garlic, cinnamaldehyde, mustard oil, or menthol, to name a few. Some other TRPs are activated by osmotic pressure, volume changes, membrane stretch or vibration. As nociceptors TRPs respond to noxious mechanical, chemical or thermal stimuli through generation of action potentials which are propagated along afferent sensory fibers to higher brain centers which generate the sensation of pain (Vay et al., 2012). TRP channel activity causes depolarization by mediating Na^+ and Ca^{2+} currents which further activate voltage-dependent ion channels. Due to their multimodal activation TRP channels can be engaged in several downstream and upstream processes accounting for positive feedback as well as for cascade boosting. In summary, TRP channels conduct signal detection, integration and amplification (Takahashi and Mori, 2011; Zheng, 2013).

TRPA1 is a member of the thermo-TRP channel family. TRPA1 is not only considered as a 'chemosensor' for pungent compounds and industrial irritants like acrolein, but also as a temperature and mechanical stress sensor (Story et al., 2003; Bandell et al., 2004; Corey et al., 2004; Jordt et al., 2004; Bautista et al., 2005, 2006; Macpherson et al., 2005). TRPA1 activation depends on reversible covalent modification of cysteine residues which arrange binding to electrophilic ligands (Hinman et al., 2006; Macpherson et al., 2007) and their activity is enhanced by increasing intracellular Ca^{2+} levels (Jordt et al., 2004). TRPA1 also mediates nociception evoked by bradykinin (Bandell et al., 2004; Jordt et al., 2004) and TRPA1 knockout mice show an impaired inflammatory pain sensation (Bautista et al., 2006).

In a study by Bang et al. (2007) the authors argue that thermo-TRP channels in general and TRPA1 channels in particular are possible targets triggering acetaldehyde-evoked pain, since acetaldehyde is responsible for the typical unpleasant and painful hang over symptoms, like headache. Using a HEK293T (human embryonic kidney) cell heterologous expression system and cultured mouse trigeminal neurons it could be shown that externally applied acetaldehyde activates mouse and human TRPA1. The half maximal effective

concentration was much lower for human TRPA1 (76 µM) than for mouse TRPA1 (1.19 mM). These findings were inferred from Fluo-3-calcium imaging studies indicating an elevation of intracellular Ca^{2+} in response to extracellularly applied acetaldehyde (1 mM). In electrophysiological experiments 1 mM external acetaldehyde evoked an increase of an outwardly rectifying current. Both effects were inhibited by the specific TRP channel blocker, camphor, the non-specific TRP channel blocker, ruthenium red, and gadolinium. Moreover, both ethanol (~170 mM) and acetic acid (10 mM) failed to evoke an intracellular Ca^{2+} response. Except TRPA1, all other representatives of the thermo-TRP channel family (TRPV1-4, TRPM8) were not affected by acetaldehyde. Interestingly, prostaglandins (PGE2), known as inflammatory mediators, were able to potentiate TRPA1 response to acetaldehyde, probably via a PLC (phospholipase C) and PKC dependent signaling pathway. Pain behavioral assays approved this results obtained from *in vitro* experiments. Intradermal injections of acetaldehyde to hindpaws of mice induced nociceptive behaviors like licking and flicking. Additional PGE2 application enhanced the behavioral effects, whereas pretreatment with TRPA1 blockers prevented acetaldehyde-induced acute pain reaction. In summary, the study showed that within the group of sensory neuronal TRP channels TRPA1 is exclusively activated by acetaldehyde and that this activation mediates acetaldehyde-evoked pain sensation (Bang et al., 2007).

CONCLUSION

Although studies were undertaken in order to reveal molecular mechanisms of acetaldehyde action basic knowledge concerning the impact of acetaldehyde on ion channels is still fragmentary. Due to its chemical and physical properties experimental application of acetaldehyde is difficult. Since it is highly reactive and volatile at room temperature it is arguable whether acetaldehyde reaches its target in sufficient concentrations. Depending on the experimental setting acetaldehyde might evaporate quickly or react with other materials such as tubes or pipettes (Mascia et al., 2001). Quantification of acetaldehyde concentrations in both blood and brain after alcohol intake is difficult due to limited *in vivo* measurement techniques. An additional problem is the rapid conversion of acetaldehyde by ADH back to ethanol and by ALDH to acetate in liver and brain. In this respect experimental and methodical restrictions are at least in part responsible for the low yield in experimental studies and some inconsistent results.

It is still a topic of controversial discussion whether significant acetaldehyde levels accumulate in brain after alcohol ingestion (Deng and Deitrich, 2008; Correa et al., 2011). For this reason an involvement of acetaldehyde in the central effects of ethanol is often disputed. Many studies favor the "Ethanol Model", others confirm the "Full Prodrug Model". From our point of view the "Modulation Model" appears the most suitable alternative to explain the controversial results of many studies and it is in line with the current state of alcohol research. Certainly, all three alternative models may coexist depending on the specific research task in focus (Quertemont and Didone, 2006).

It is not conclusively clarified whether acetaldehyde is able to diffuse freely into or out of the cell in effective amounts per unit time. Nevertheless, in the majority of the studies acetaldehyde was applied to the extracellular face of the cell membrane. Since the key actors of the ethanol metabolism - ALDH, CYP2E1, and catalase - are present in the cytosol, acetaldehyde is enzymatically produced within cells presumably having its main targets on the intracellular sites. Therefore, it is to be expected that acetaldehyde performs its action on ion channels from the intracellular face of the plasma membrane. In fact, all studies presented in this review which applied acetaldehyde to the cytosolic side of the membrane reported an acetaldehyde-mediated effect on ion channels. In contrast, many of the studies using external application of acetaldehyde failed to reveal acetaldehyde actions on ion channels. In some cases extracellular acetaldehyde-mediated effects still occurred, but only at very high and physiologically not relevant concentrations. Interestingly, there are also a few studies showing extracellular acetaldehyde action on ion channels at low concentrations in the µM - and even in the nM range. These results suggest that acetaldehyde may either cross the cell membrane in the particular experimental approaches, or interact with extracellular components of the respective channels. Regarding membrane permeability/transport of acetaldehyde the different durations of experiments (time allowed for permeation) may play a role and be responsible for some inconsistent outcome. Moreover, in several studies behavioral effects, including aversive, sedative, amnesic, hypnotic or reinforcing properties, were induced by acetaldehyde applied intraperitoneal or intravenous (for review see Quertemont et al., 2005b). This may be an indication for acetaldehyde being able to cross membranes and eventually enter the brain by passing the blood brain barrier. However, since the effects reported by Quertemont et al. (2004, 2005b) only occurred at relatively high acetaldehyde doses (100-300 mg/kg body weight), it appears questionable if acetaldehyde from physiological

peripheral sources can reach the brain in sufficient concentrations to produce the central effects. Hence, although some possible impact of extracellular acetaldehyde in the periphery as well as in the brain cannot be excluded its relevance under physiological conditions is still a matter of debate.

Only a few studies consider the fact that under physiological conditions acetaldehyde and ethanol are simultaneously present in tissues and cells. Mascia et al. (2001), who investigated the effect of acetaldehyde on several ligand gated ion channels as well as on GIRK2 and DAT, also tested for interactions with ethanol. However, ethanol action on these proteins appeared not to be influenced by the simultaneous presence of acetaldehyde. In contrast studies from our lab clearly indicate mutual interactions of acetaldehyde and ethanol on BK channels. In this respect the side of application of acetaldehyde to the membrane turned out to be a determining factor. If applied to the intracellular side acetaldehyde did not only countervail the ethanol mediated activation at BK channels (Handlechner et al., 2013), but even reduced BK channel activity well below basic levels during long-term co-application with ethanol (Handlechner et al., 2014). These findings provide evidence that the impact of acetaldehyde and ethanol should be considered in combination since the effects due to mutual interactions could be more substantial than those of the individual compounds. More research is needed particularly concerning those ion channels that have been found to be targeted by alcohol but so far have not been studied in the context with acetaldehyde.

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