

## Chapter 2

# Cellular Mechanisms in Acupuncture Points and Affected Sites

Wolfgang Schwarz and Quanbao Gu

**Abstract** The objective of our work is the elucidation of mechanisms underlying initiation, transmission and the final effects of acupuncture and moxibustion. In this chapter, we shall focus mainly on possible cellular events in tissue affected by Chinese medicine treatments using basically electrophysiological techniques combined with molecular biological and radioactive tracer techniques to measure activity of transmembrane transport.

In the first part we will review work of our laboratory suggesting that acupuncture-induced pain suppression involves interaction of  $\delta$ -opioid receptor (DOR) with the neurotransmitter transporters for glutamate (EAAC1),  $\gamma$ -amino butyric acid (GAT1) and the sodium pump ( $\text{Na}^+$ ,  $\text{K}^+$ -ATPase). Reduced activity of the transporters by co-expression of DOR resulted from intermolecular interaction with DOR. In addition, EAAC1 became stimulated in response to DOR activation, while GAT1 became inhibited. The  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase was not affected by DOR activation, but higher sensitivity to DOR agonist was found in response to sodium pump stimulation. Since endorphins are released in response to acupuncture, the effects described here may contribute to acupuncture-induced pain suppression.

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W. Schwarz (✉)

Laboratory of Cellular Electrophysiology, Shanghai Research Center for Acupuncture and Meridians, Shanghai 201203, China

Institute for Biophysics, Goethe University Frankfurt am Main,  
Max-von-Laue-Str. 1, 60438 Frankfurt am Main, Germany  
e-mail: wolfgang.schwarz@email.de

Q. Gu

Laboratory of Cellular Electrophysiology, Shanghai Research Center for Acupuncture and Meridians, Shanghai 201203, China  
e-mail: guquanbao@126.com

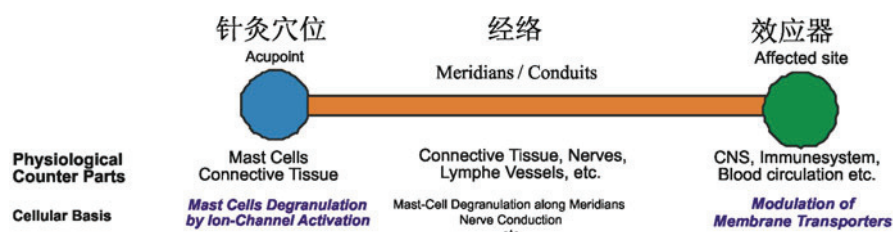
In the second part we will review work on effects of drugs on membrane transporters. In treatment of asthmatic rats by acupuncture cyclophilin A (CyPA) becomes over-expressed. Release of airway smooth muscle contraction by CyPA-induced inhibition of  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  exchanger may be the underlying mechanism. As an example for effects of Chinese herb extracts on the transporters described in Sect. 2.1, we will review the effect of *Acorus* extract on EAAC1 function showing that  $\alpha$ -asarone effectively inhibits EAAC1-mediated current but stimulates glutamate transport. These effects may contribute to reduced excitatory activity supplementary to acupuncture.

Conclusion: The generally accepted effect of acupuncture is pain relief. We suggest that modulation of central nervous synaptic activity may be involved through indirect modulation of the activity of neurotransmitter transporters by endorphins. Membrane transporters may also be the target for drugs of Chinese herbs and for endogenous acupuncture-induced “drugs.”

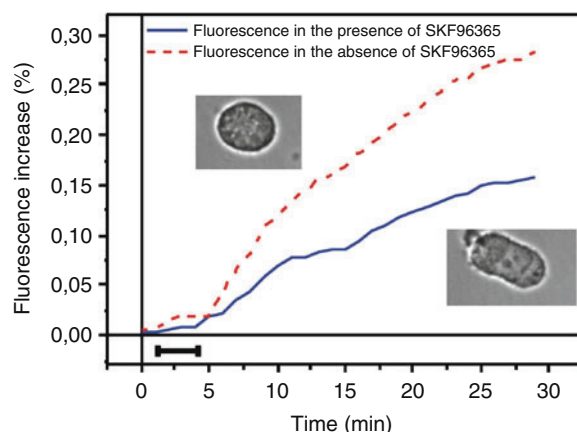
**Keywords** Neurotransmitter transporter • Opioid receptor • Na pump •  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  exchanger • *Xenopus* oocyte expression system • Mast cell • Electrophysiology

## 2.1 Introduction

Acupuncture/moxibustion but also treatments with Chinese herbal formulae are methods of Traditional Chinese Medicine. Stimulation of organ-specific acupuncture points (acupoints), located close to the body surface, leads to signals that seem to spread along a network of conduits beneath the so-called meridians to the affected sites (compare Fig. 2.1). Chinese medicine treatments may interfere with all of these structures. From the point of view of Western medicine, we are interested in the physiological and cellular counterparts of acupoints, conduits and affected sites. In our previous work, our laboratory has focused on exploration of cellular events occurring in acupoints (Zhang et al. 2008; Wang et al. 2010) as well as in affected sites (Xia et al. 2006; Deng et al. 2009).



**Fig. 2.1** Simplified scheme for underlying structures and mechanisms in acupuncture effects. Some examples of possible physiological and cellular counterparts are listed



**Fig. 2.2** Noxious heat (53°C) induced intracellular  $\text{Ca}^{2+}$  increase and associated mast-cell degranulation. Relative Calcium Green-1 AM (4 mM) fluorescence increase in HMC1 mast cells is shown after subtraction of background fluorescence. The *bar* indicates the time period of heat application. A selected cell before (*upper left inset*) and 20 min after the heat application (*lower right inset*) illustrates the heat-induced degranulation. [Zhang, D., Spielmann, A., Wang, L., Ding, G.H., Gu Q.B., Schwarz, W. Activation of mast-cell degranulation by different physical stimuli involves activation of transient-receptor-potential channel TRPV2 (unpublished)]

It seems quite established by now that degranulation of mast cells (Fig. 2.1) in response to mechanical stimulation of acupoints is a prerequisite for acupuncture-induced pain suppression in rat (Zhang et al. 2008). Also stimulation by noxious heat, as applied during moxibustion, or by red laser light, as applied in laser acupuncture, leads to mast-cell degranulation (Zhang et al. 2012).

In particular for the human mast-cell line HMC1, the transient potential receptor (valinoide-sensitive isoform 2), the TRPV2, had been demonstrated to be activated by these physical stimuli and to contribute to the mast-cell degranulation (Zhang et al. 2012). It could be shown that also activation of additional ion-selective channels that facilitate  $\text{Ca}^{2+}$  entry forms the basis for the mast-cell degranulation (Zhang et al. 2012; Wang et al. 2010).

Figure 2.2 shows the increase of intracellular  $\text{Ca}^{2+}$  in response to heat application using Calcium Green-1 AM as a  $\text{Ca}^{2+}$  indicator.<sup>1</sup> Part of the  $\text{Ca}^{2+}$  increase can be blocked by the TRPV2 channel blocker SKF96365. The two micrographs in Fig. 2.2 show the same cell before and 20 min after heat application. Similar results were obtained for a  $\text{Cl}^-$ -permeable channel (Wang et al. 2010). Events occurring in acupoints are also dealt with in another chapter of this book by Ding et al. (Chap. 3).

<sup>1</sup> Zhang, D., Spielmann, A., Wang, L., Ding, G.H., Gu Q.B., Schwarz, W. Activation of mast-cell degranulation by different physical stimuli involves activation of transient-receptor-potential channel TRPV2 (unpublished)

Communication of mast cells with nerve endings is well known (Bauer and Razin 2000) and may form the next subsequent step within the acupoints for transmission along the conduits of the acupuncture stimuli to the affected organ (Fig. 2.1). This step is subject to current investigations in our laboratory. In this chapter we now like to review some of our speculations on acupuncture-induced effects in organ tissue. In Sect. 2.2 we will focus on molecular events that could contribute in the central nervous system (CNS) to acupuncture-induced pain suppression; this will be, in addition to neurotransmitter transporters, the  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase. In Sect. 2.3 we like to present some data on effects of selected drugs on membrane transporters. As an example of an endogenous “drug,” we will present data showing a protein becoming over-expressed after acupuncture treatment of asthmatic rat inhibits  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  exchanger. In addition, we will show by another example that the transporters dealt with in Sect. 2.2 as target for acupuncture may also be a target for extracts of Chinese herbs.

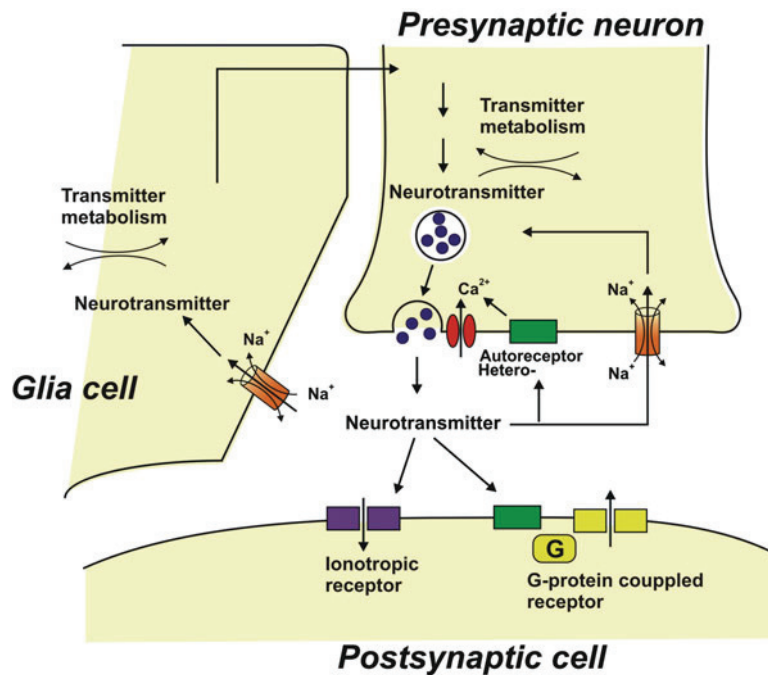
## 2.2 Possible Role of Membrane Transporters in Pain Suppression by Acupuncture

Neurotransmitter transporters play a key role in the regulation of synaptic transmission (for illustration see Fig. 2.3). On the arrival of an action potential at a nerve terminus of the presynaptic neuron, neurotransmitter is released in response to  $\text{Ca}^{2+}$  entry. The transmitter will subsequently bind to and activate ionotropic and/or G-protein-coupled receptors at the postsynaptic membrane. This will result in depolarisation or hyperpolarisation of the membrane, depending on whether an excitatory or inhibitory transmitter, respectively, is released at the particular synapse. To terminate synaptic transmission, the neurotransmitter needs to be removed, and this is achieved by highly efficient,  $\text{Na}^+$ -gradient-driven neurotransmitter transporters in the presynaptic neuron and surrounding glia cells.

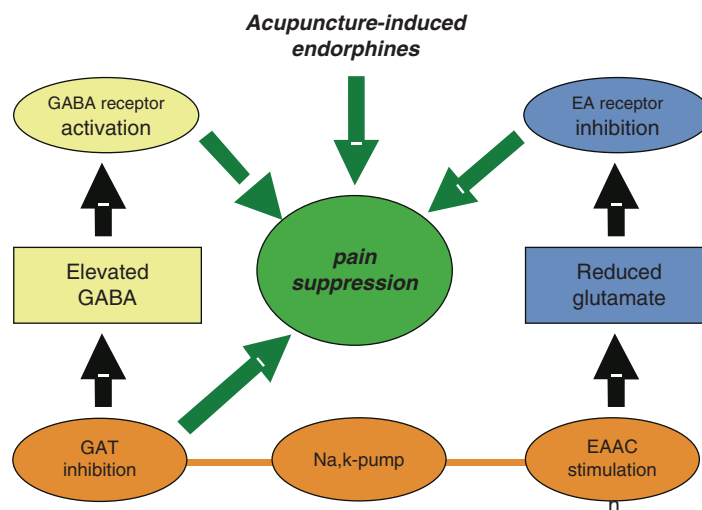
Glutamate and GABA are the dominating excitatory and inhibitory neurotransmitters in the mammalian brain, respectively. The predominate transporters controlling glutamate and GABA in the CNS are the excitatory neurotransmitter transporter EAAC1 (or also named EAAT3) and the GABA transporter GAT1.

It is generally accepted that pain sensation can be suppressed by acupuncture and that regulation of the glutamatergic and the GABAergic systems is involved in pain sensation. It could be demonstrated that inhibition of excitatory amino acid (EA) receptors resulted in pain suppression (Zhang et al. 2002). Reduction of EA-receptor activity may also be achieved by reduced glutamate concentration in the synaptic cleft, and reduction of glutamate concentration can be achieved by stimulating EAAC activity (compare Fig. 2.4).

In analogy to stimulation of EAAC1, we may expect for the GABAergic system that inhibition of the GABA transporter will result in elevation of GABA concentration in the synaptic cleft and hence in stimulation of GABA receptor activity (see Fig. 2.4); this could contribute to increased inhibitory synaptic transmission and also to reduced pain sensation. Indeed, experiments with transgenic mice with knockout or over-expressed GABA transporters GAT1 have demonstrated that the GAT1 is correspondingly involved in pain sensation (Hu et al. 2003).



**Fig. 2.3** Schematic illustration of processes at a synapse. The release of neurotransmitter from the presynaptic nerve terminus is initiated by the activation of  $\text{Ca}^{2+}$  channels. The neurotransmission is based on the subsequent activation of postsynaptic receptors. Neurotransmission is terminated by  $\text{Na}^+$ -driven neurotransmitter transporters



**Fig. 2.4** Illustration of possible involvement of glutamatergic and GABAergic pathways in pain suppression. It was already demonstrated that inhibition of excitatory amino acid (EA) (Zhang et al. 2002) and reduced GAT1 activity (Hu et al. 2003) result in reduced pain sensation. Based on the knowledge that acupuncture-induced release of endorphins is involved in pain suppression, we may speculate that acupuncture interferes with these two neuronal systems via activation of opioid receptors

It was shown previously (Han 2004) that acupuncture leads to activation of enkephalinergic neurons and release of endogenous morphine, the endorphins. The question, therefore, may arise whether opioids can modulate the activity of the transporters for the excitatory neurotransmitter glutamate and the inhibitory neurotransmitter GABA, EAAC1 and GAT1, respectively. In this respect the activity of the  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase should not be neglected since this ATP-driven pump controls the  $\text{Na}^+$  gradient across the cell membrane, and the  $\text{Na}^+$  gradient is driving force for the neurotransmitter transporters.

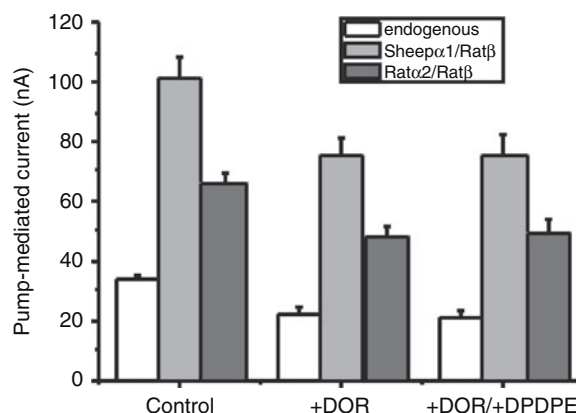
To investigate possible interference of enkephalinergic receptors and the membrane transport proteins, *Xenopus* oocytes can be used as a model system to measure the activity of membrane transporters (Schwarz 2001). The advantage of this model system compared to the native cell is that function of the respective transport protein can be studied independent of the interference with other neuronal membrane proteins. With heterologously co-expressed opioid receptors, influence of the receptor on the activity of one of the neurotransmitter transporters or the sodium pump can be characterised, e.g. without interference with other components of the glutamatergic or GABAergic system. For detecting the activity of the respective transport protein, steady-state currents mediated by these electrogenic transporters can be recorded in voltage-clamp experiments (Schwarz 2001). The  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase pumps 3  $\text{Na}^+$  ions out of the cell and 2  $\text{K}^+$  ions into the cell [see, e.g. (Schwarz and Gu 1988)] per ATP molecule split and hence generates outward-directed net current. The neuronal glutamate transporter EAAC1 transports for 1 glutamate 3  $\text{Na}^+$  ions together with 1  $\text{H}^+$  into the cell and 1  $\text{K}^+$  out of the cell [see, e.g. (Zerangue and Kavanaugh 1996)], and hence generates inward-directed net current. The GABA transporter GAT1 transports for 1 GABA 2  $\text{Na}^+$  ions and 1  $\text{Cl}^-$  into the cell [see, e.g. (Hilgemann and Lu 1999; Kavanaugh et al. 1992; Keynan and Kanner 1988; Krause and Schwarz 2005)], and hence also generates inward-directed net current. These currents can be measured as steady-state currents under voltage clamp and can serve as a measure for transporter activity. The neurotransmitter transporters can also operate in ion-selective channel modes without transporting the glutamate (Wadiche et al. 1995; Slotboom et al. 2001) or GABA [see, e.g. (Risso et al. 1996; Eckstein-Ludwig et al. 2000; Krause and Schwarz 2005)]. These currents contribute to the total current mediated by the respective transporter.

In addition to the electrical detection of the activity of the transporter, radioactive tracer transport measurements can be performed. For the neurotransmitter transporters discussed in this overview, we will describe in addition to results on electrical measurements also results on uptake measurements of  $^3\text{H}$ -labelled glutamate [see, e.g. (Xia et al. 2006)] and GABA [see, e.g. (Krause and Schwarz 2005)], respectively.

### **2.2.1 Interaction of the $\text{Na}^+$ , $\text{K}^+$ Pump and Co-expressed $\delta$ -Opioid Receptors (Deng et al. 2009)**

Expression of  $\delta$ -opioid receptor (DOR), but not other opioid receptors, resulted in reduced activity of the endogenous sodium pump in *Xenopus* oocytes [Fig. 2.5

**Fig. 2.5** Effect of co-expressed DOR on sodium pump current. Results are from three different isoforms and at  $-60$  mV in *Xenopus* oocytes, the endogenous pump and heterologously expressed sheep and rat pumps. Data represent means  $\pm$  SEM from 3 to 14 oocytes (based on data presented by Deng et al. 2009)



(open bars)]. Stimulation of DOR by 100 nM of the DOR agonist [D-Pen2,5]-encephalin (DPDPE) had no pronounced additional effect on pump activity. Qualitatively similar results were obtained for exogenously co-expressed sodium pumps (e.g. *Sheep*  $\alpha$ 1/*Rat*  $\beta$ 1 or *Rat*  $\alpha$ 2/*Rat*  $\beta$ 1, see Fig. 2.5).

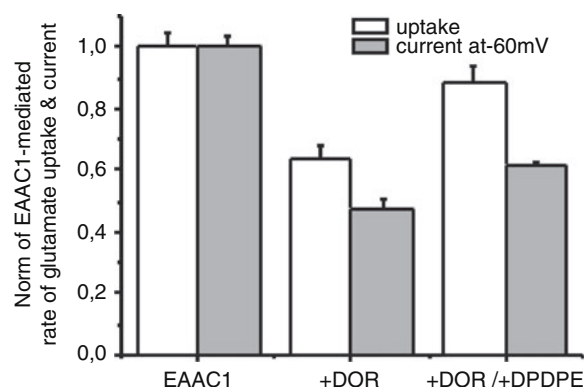
The authors suggested that the reduction of pump activity in response to co-expression of DOR resulted from direct protein–protein interaction. Co-localisation of DOR and sodium pump could be demonstrated not only in the model system *Xenopus* oocytes but also in hippocampal neurons of rat by co-immunoprecipitation suggesting the physiological significance of the interaction of DOR with sodium pump. The reported reduction of sensitivity of DOR to the agonist DPDPE in response to pump activation (Deng et al. 2009) was also attributed to this interaction; for the *Sheep*  $\alpha$ 1/*Rat*  $\beta$  pump, e.g. the apparent affinity for DPDPE decreased from  $5.9 \pm 0.6$  to  $3.7 \pm 0.5 \mu\text{M}^{-1}$  in response to activation of the sodium pump. To which extent the change in affinity has physiological significance needs further investigation.

### 2.2.2 Interaction of the Glutamate Transporter EAAC1 and Co-expressed $\delta$ -Opioid Receptors (Xia et al. 2006)

DOR co-expressed with the neuronal glutamate transporter EAAC1 in *Xenopus* oocytes, but not  $\mu$ -opioid receptor, down-regulated EAAC1 function, and stimulation of DOR by 100 nM DPDPE could counteract the down-regulated function of EAAC1, as shown for glutamate uptake into *Xenopus* oocytes in Fig. 2.6.

As for the  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase and DOR, direct interaction of EAAC1 and DOR was suggested. Co-localisation of the two membrane proteins could be demonstrated by immunofluorescence microscopy and co-immunoprecipitation in the *Xenopus* oocytes. This could also be demonstrated in hippocampal neurons of rat supporting the physiological significance of the DOR–EAAC1 interaction. As for the reduced sodium pump activity, reduced EAAC1 function may be the result of the direct





**Fig. 2.6** Effect of co-expressed DOR on EAAC1-mediated uptake and current. DOR and EAAC1 were heterologously expressed in *Xenopus* oocytes. Data are normalised to uptake/current at  $-60$  mV without co-expressed DOR and represent means  $\pm$  SEM of at least 20 oocytes for uptake and 12 oocytes for current measurements (based on data presented by Xia et al. (2006))

interaction. The increased EAAC1 activity in response to activation of DOR may be the result of release of the inhibitory DOR–EAAC1 interaction.

### 2.2.3 Interaction of the GABA Transporter GAT1 and Co-expressed $\delta$ -Opioid Receptors

Co-expression of DOR with GAT1 led to a reduction in the number of fully functional transporters though the total number of expressed transporter was not changed as could be demonstrated by analysing transient charge movements by GAT1 and Western blot.<sup>2</sup>

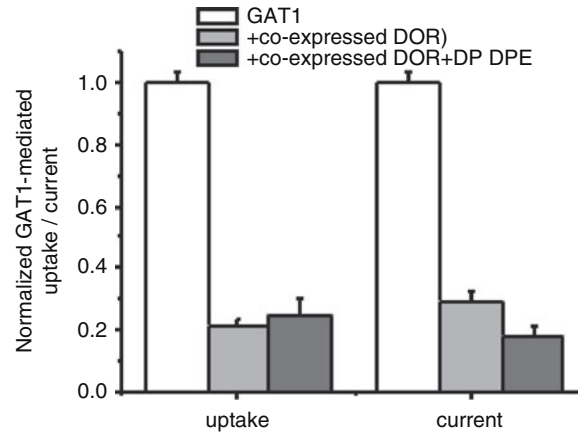
In addition to this reduction in the number of functional transporters, rate of substrate translocation became dramatically reduced; also the current mediated by GAT1 became reduced by similar extent (Fig. 2.7). Activation of DOR by 100 nM DPDPE had no significant effect on rate of uptake, but current became slightly, but significantly, reduced.

In preliminary experiments,<sup>3</sup> inhibition of GAT1-mediated current in response to DOR activation could also be demonstrated in rat brain slices. The midbrain periaqueductal gray (PAG) is an important part of the CNS for controlling analgesia. Figure 2.8 shows the current mediated by GAT1 from a single neuron in a PAG slice of rat. Application of 100 nM DPDPE could completely block the current.

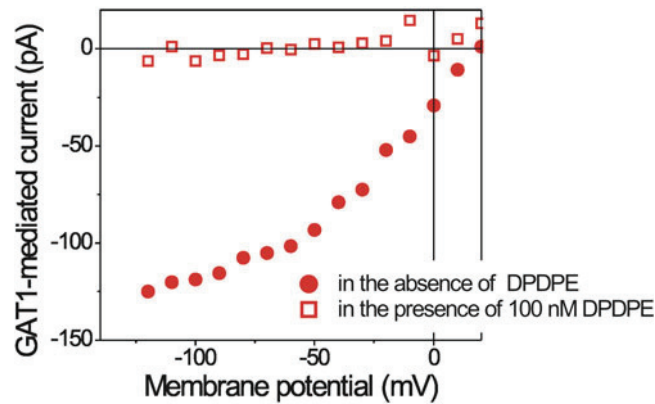
<sup>2</sup> Pu L., Xia, P., Fücke, T., Gu, Q.B., Pei, G., Schwarz, W. Regulation of GABA transporter by expression and activation of  $\delta$ -opioid receptor (unpublished)

<sup>3</sup> Ren S.L., Gu, Q.B., Ding, G.H., Schwarz, W. Modulation of GAT1-mediated current in PAG neuron by DOR activation (unpublished)





**Fig. 2.7** Effect of co-expressed DOR on GAT1-mediated uptake and current. Data represent averages of 38–71 oocytes for uptake measurements and at least 6 oocytes for the current measurements at  $-60$  mV. [Pu L., Xia, P., Fücke, T., Gu, Q.B., Pei, G., Schwarz, W.: Regulation of GABA transporter by expression and activation of  $\delta$ -opioid receptor (unpublished)]



**Fig. 2.8** Effect of DOR activation by DPDPE on GAT1-mediated current in rat brain slice from PAG area. Whole-cell patch-clamp recordings. The GAT1-mediated current was determined as Tiagabine-sensitive steady-state current. Presented data are from a single experiment, but similar results were obtained in four other PAG slices. [Ren S.L., Gu, Q.B., Ding, G.H., Schwarz, W. Modulation of GAT1-mediated current in PAG neuron by DOR activation (unpublished)]

In conclusion, we suggest that DOR can specifically and directly interact with a variety of membrane transport systems including the  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase, EAAC1 and GAT1. This interaction leads for all three transport systems to reduced transport. The sodium pump not only affects neuronal activity directly but also modulates through functional interaction with DOR the sensitivity of DOR to agonist, and hence sodium pump modulation may also modulate pain sensation. For GAT1,

activation of DOR leads to inactivation of GAT1-mediated current; in PAG brain slices the current could even completely be blocked indicating complete inhibition of GAT1 activity in response to DOR activation. The stimulation of EAAC1 by DOR activation, via release of the inhibitory DOR–EAAC1 interaction, is opposite to the GAT1 inhibition. These effects of activation of DOR with GAT1 inhibition and EAAC1 stimulation would fit to the idea of acupuncture-induced pain suppression as illustrated in Fig. 2.4. In addition to the interaction of DOR with membrane transporters, the interaction between DOR and voltage-gated  $\text{Na}^+$  channels had been suggested and is also dealt with in Chap. 6.

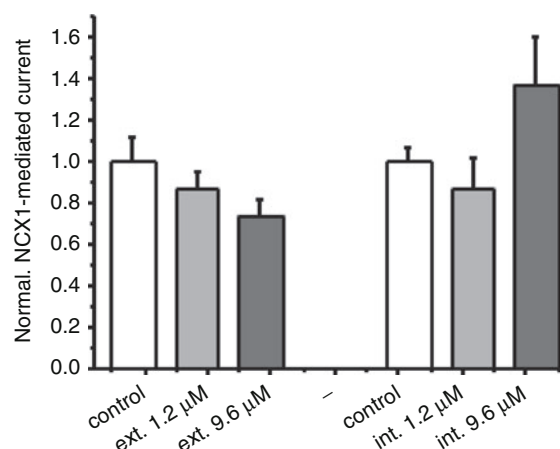
## 2.3 Effects of Drugs on Membrane Transporters Modulated by Acupuncture

In the previous section we have described that membrane transport systems can be a target for acupuncture stimuli by interaction with opioid receptors that may become activated by acupuncture-induced elevation of endorphins. In this second section we will report that membrane transporters can also be targets for drugs, endogenous acupuncture-induced drugs that act on membrane transporter as well as components of Chinese medicine extracts.

### 2.3.1 *CyPA as an Acupuncture-Induced Inhibitor of $\text{Na}^+$ , $\text{Ca}^{2+}$ Exchanger*

In treatment of asthmatic rats by acupuncture, a protein could be identified that became over-expressed (Wang et al. 2009b). This protein turned out to be cyclophilin A (CyPA). If CyPA were involved in relief from asthma, we may ask for the target CyPA interacts with. Airway smooth muscle (ASM) cells have been an important target for asthma treatment in the past using drugs that interact with various plasma membrane receptors. Since smooth muscle contraction is governed by cytosolic  $\text{Ca}^{2+}$  ( $\text{Ca}_i^{2+}$ ), control and modulation of  $\text{Ca}_i^{2+}$  are essential for the status of ASM cell contraction. Hence, all pathways involved in  $\text{Ca}_i^{2+}$  regulation may be likely candidate targets (for a review see Janssen 2009), in particular also for CyPA. An important protein involved in the regulation of  $\text{Ca}_i^{2+}$  is the  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  exchanger (NCX) that operates in a  $3\text{Na}^+:1\text{Ca}^{2+}$  stoichiometry. In its normal mode, NCX uses the inward-directed gradient for  $\text{Na}^+$  to remove  $\text{Ca}^{2+}$  from the cytoplasm. NCX can also operate in a reversed mode (Hirota et al. 2007) pumping  $\text{Ca}^{2+}$  into the cytoplasm. Muscle contraction is governed by release of  $\text{Ca}^{2+}$  from the sarcoplasmic reticulum (SR), and the refilling depends on the availability of  $\text{Ca}_i^{2+}$ . The reversed mode of the NCX of the plasma membrane has been demonstrated to contribute to refilling the  $\text{Ca}^{2+}$  stores in ASM cells (Hirota et al. 2007).

**Fig. 2.9** Effect of externally applied and injected hCyPA on NCX1-mediated current. Data represent mean inward currents of 7–10 oocytes ( $\pm$ SEM) and were normalised to  $-60$  mV. [Wang, Y., Gu, Q.B., Yang, Y.Q., Schwarz, W. (2009) Effects of human Cyclophilin A on  $\text{Na}^+/\text{Ca}^{2+}$  exchanger NCX1 in *Xenopus* oocytes (unpublished)]



To investigate modulation of NCX, *Xenopus* oocytes with heterologously expressed NCX1 were used as model system. NCX1-mediated current was determined as the current component sensitive to  $2 \text{ mM Ni}^{2+}$  in the external solution. Oocytes not expressing NCX1 did not exhibit  $\text{Ni}^{2+}$ -sensitive currents.

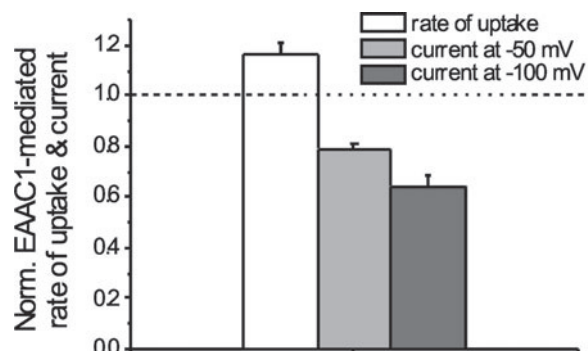
Figure 2.9 shows the effect of externally applied (overnight incubation) in  $1.2\text{--}9.6 \mu\text{M}$  human CyPA (hCyPA) at  $-60 \text{ mV}$ . The current became slightly, but significantly, reduced after the incubation with the hCyPA (Fig. 2.9).

If hCyPA were injected into *Xenopus* oocytes (final internal concentration up to  $4.8 \mu\text{M}$ ), NCX1-mediated current was hardly affected. Only at very high amounts (final internal concentration more than  $9 \mu\text{M}$ ), current became elevated (Fig. 2.9). In whole human blood sample hCyPA of about  $200 \text{ nM}$  has been reported, but in plasma only a very small fraction, if any, could be detected (Allain et al. 1995). On the other hand, in patients with inflammations cytoplasmic CyPA of several hundred nanometres has been found (Billich et al. 1997; Jin et al. 2004). Therefore, the inhibitory effect of CyPA on the activity of NCX1 may have physiological, therapeutic relevance. It has been reported (Hirota et al. 2007) that in ASM cells the reversed mode of NCX provides a source of  $\text{Ca}^{2+}$  for store refilling. Inhibition of NCX by acupuncture-induced CyPA elevation may, therefore, counteract the refilling and hence favour relaxation of the smooth muscle cells.

### 2.3.2 Effects of $\alpha$ -Asarone on Membrane Transport

In Chinese Medicine acupuncture is usually supplemented by treatment with Chinese herbal formula. The membrane proteins, which have been investigated in our laboratory as putative targets for acupuncture effects, may also be targets for such herbal extracts. In our laboratory, the *Xenopus* oocytes served also as an ideal model system for drug screening. As in the previous section, voltage-clamp and

**Fig. 2.10** Rate of glutamate uptake and glutamate-induced current in *Xenopus* oocytes expressing EAAC1. At 200  $\mu\text{M}$   $\alpha$ -asarone,  $^3\text{H}$ -glutamate uptake is stimulated, but glutamate-induced current is inhibited potential dependently (based on data presented by Gu et al. (2009))



tracer-flux experiments were performed to detect modulation of the  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase and the neurotransmitter transporters. As an example we will illustrate the effect of  $\alpha$ -asarone on the glutamate transporter EAAC1 (Gu et al. 2009).

Different extracts of rhizomes of *Acorus tatarinowii* were tested with respect to their effects on the sodium pump, EAAC1 and GAT1. As the only effective component,  $\alpha$ -asarone could be identified as modulator of EAAC1 activity; GAT1 and the  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase were not affected.

Figure 2.10 illustrates that glutamate uptake is stimulated by 200  $\mu\text{M}$   $\alpha$ -asarone by about 15 %, while EAAC1-mediated current is inhibited with slight voltage dependence (stronger inhibition at more negative potentials). This voltage dependence reflects that  $\alpha$ -asarone inhibits the  $\text{Cl}^-$ -channel mode of EAAC1, which contributes to total EAAC1-mediated current with more negative potentials.

It has been reported that glutamatergic nerve terminals accumulate intracellularly  $\text{Cl}^-$  (Price and Trussell 2006). As a consequence, inhibition of  $\text{Cl}^-$  channels will hyperpolarise the membrane potential. Inhibition of the EAAC1-mediated  $\text{Cl}^-$ -channel mode by  $\alpha$ -asarone will, therefore, contribute to stabilising the membrane potential and, together with the stimulated rate of glutamate uptake, counteract excitatory synaptic transmission. This could contribute to the reported anticonvulsive (Wang et al. 1998; Yang et al. 2006) and neuroprotective (Cho et al. 2002) effect of  $\alpha$ -asarone.

At similar concentrations, in the range of 100  $\mu\text{M}$ ,  $\alpha$ -asarone inhibits also the purinergic receptor  $\text{P2X}_7$  that is activated by ATP exceeding 100  $\mu\text{M}$  concentrations (Spielmann et al. 2008). Activation of  $\text{P2X}_7$  receptors has been shown to facilitate pro-inflammatory processes associated with arthritis (Dubyak and El-Motassim 1995; Carroll et al. 2009), and within the nervous system, pro-inflammatory processes may contribute to development and maintenance of chronic pain (Carroll et al. 2009; Hughes et al. 2007). In addition to the above described effect of  $\alpha$ -asarone on EAAC1, the inhibition of  $\text{P2X}_7$  could contribute to the previously reported observation that  $\alpha$ -asarone can protect neuronal cells from excitotoxic cell death (Cho et al. 2002).

## 2.4 Concluding Remarks

The work of our laboratory suggests that the generally accepted effect of acupuncture in pain relief can at least partially be mediated by modulation of central nervous synaptic activity. This modulation may involve indirect modulation of GABA and glutamate transporter activity by acupuncture-induced release of endorphins. Since modulation of  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity can affect opioid sensitivity, this membrane protein should be considered as an interesting target in various kinds of treatment. Membrane transporters may also be a direct target for acupuncture-activated endogenous drugs as illustrated for the effect of hCypA on the  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  exchanger in treatment of asthma. The membrane transport systems that can be affected by acupuncture should also be considered as target for Chinese drugs.

In conclusion dysfunction of neurotransmitter transporters is involved in various neurological disorders, like the GABA transporter in epilepsy [see, e.g. (Fueta et al. 2003; Conti et al. 2004)] or the glutamate transporter in excitotoxicity [e.g. (Li et al. 1997; Maragakis and Rothstein 2004; Sheldon and Robinson 2007)]. Therefore, these transporters have become important target for Western medicines but may also be targets for curing neuronal diseases by acupuncture.

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## References

- Allain F, Boutillon C, Mariller C, Spik G (1995). Selective assay for CyPA and CyPB in human blood using highly specific anti-peptide antibodies. *J Immunol Meth* 178:113–120.
- Bauer O, Razin E (2000). Mast Cell-Nerve Interactions. *News Physiol Sc* 15:213–218.
- Billich A, Winkler G, Aschauer H, Roth A, Peichl P (1997). Presence of cyclophilin A in synovial fluids of patients with rheumatoid arthritis. *J Exp Med* 185:975–980.
- Carroll WA, Donnelly-Roberts D, Jarvis MF (2009). Selective P2X7 receptor antagonists for chronic inflammation and pain. *Purinergic Signal* 5:63–73.
- Cho J, Kim YH, Kong JY, ang CH, ark CG (2002). Protection of cultured rat cortical neurons from excitotoxicity by asarone, a major essential oil component in the rhizomes of *Acorus gramineus*. *Life Sci* 71:591–599.
- Conti F, Minelli A, Melone M (2004). GABA transporters in the mammalian cerebral cortex: localization, development and pathological implications. *Brain Res Rev* 45:196–212.
- Deng HP, Yang ZJ, Li YT, Bao GB, Friedrich T, Gu QB, Schwarz W (2009). Interaction of  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase and co-expression of  $\delta$ -opioid receptor. *Neurosc Res* 65:222–227.
- Dubyak GR, El-Motassim C (1995). Signal transduction via P2-purinergic receptors for extracellular ATP and other nucleotides. *Am J Physiol* 265:C577–C606.

- Eckstein-Ludwig U, Fueta Y, Fei J, Schwarz W (2000). The neuronal GABA transporter GAT1 as a target for action of antiepileptic drugs. In: Suketa Y, et al., editors. *Control and Diseases of Sodium Transport Proteins and Channels*. Amsterdam:Elsevier Press. p. 373–376.
- Fueta Y, Vasilets LA, Takeda K, Kawamura M, Schwarz W (2003). Down-regulation of GABA-transporter function by hippocampal translation products: Its possible role in epilepsy. *Neuroscience* 118:371–378.
- Gu QB, Du HM, Ma CH, Fotis H, Wu B, Huang CG, Schwarz W (2009). Effects of  $\alpha$ -asarone on the glutamate transporter EAAC1 in *Xenopus* oocytes. *Planta Med* 75:1–4.
- Han J-S (2004). Acupuncture and endorphins. *Neurosci Lett* 361:258–261.
- Hilgemann DW, Lu C-C (1999). Gat1 (GABA:Na<sup>+</sup>:Cl<sup>-</sup>) Cotransport function - database reconstruction with an alternating access model. *J Gen Physiol* 114:459–475.
- Hirota S, Pertens E, Janssen LJ (2007). The reverse mode of the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger provides a source of Ca<sup>2+</sup> for store refilling agonist-induced Ca<sup>2+</sup> mobilization. *Am J Physiol Lung Cell Mol Physiol* 292:L438–L447.
- Hu J-H, Yang N., Ma Y-H, Zhou X-G, Jiang J, Duan S-H, Mei Z-T, Fei J, Guo L-H (2003). Hyperalgesic Effects of  $\gamma$ -Aminobutyric Acid transporter I in Mice. *Journal of Neuroscience Research* 73:565–572.
- Hughes JP, Hatcher JP, Chessell IP (2007). The role of P2X7 in pain and inflammation. *Purinergic Signal* 3:163–169.
- Janssen LJ (2009). Asthma therapy: how far have we come, why did we fail and where should we go next? *Eur Respir J* 33:11–20.
- Jin ZG, Lungu AO, Xie L, Wang M, Wong C, Berk BC (2004). Cyclophilin A is a proinflammatory cytokine that activates endothelial cells. *Arterioscler Tromb Vasc Biol* 24:1186–1191.
- Kavanaugh MP, Arriza JL, North RA, Amara SG (1992). Electrogenic uptake of  $\gamma$ -aminobutyric acid by a cloned transporter expressed in *Xenopus* oocytes. *J Biol Chem* 267:22007–22009.
- Keynan S, Kanner BI (1988).  $\gamma$ -Aminobutyric acid transport in reconstituted preparations from rat brain: Coupled sodium and chloride fluxes. *Biochemistry* 27:12–17.
- Krause S, Schwarz W (2005). Identification and Selective Inhibition of the Channel Mode of the Neuronal GABA transporter 1. *Molecular Pharmacology* 68:1728–1735.
- Li S, Mallory M, Alford M, Tanaka S, Masliah E (1997). Glutamate transporter alterations in Alzheimer disease are possibly associated with abnormal APP expression. *J Neuropathol Exp Neurol* 90:1–911.
- Maragakis NJ, Rothstein JD (2004). Glutamate transporters: animal models to neurologic disease. *Neurobiol Disease* 15:461–473.
- Price GD, Trussell LO (2006). Estimate of the chloride concentration in a central glutamatergic terminal: A Gramicidin perforated-patch study on the Calyx of Held. *J Neurosci* 26:11432–11436.
- Risso S, Defelice LJ, Blakely RD (1996). Sodium-dependent GABA-induced currents in GAT1-transfected HeLa cells. *J Physiol* 490:691–702.
- Schwarz W (2001). Electrophysiology in Carrier Research. *J Biol Membranes* 19:48–58.
- Schwarz W, Gu Q-B (1988). Characteristics of the Na<sup>+</sup>/K<sup>+</sup>-ATPase from *Torpedo californica* expressed in *Xenopus* oocytes: A combination of tracer flux measurements with electrophysiological measurements. *Biochim Biophys Acta* 945:167–174.
- Sheldon AL, Robinson MB (2007). The role of glutamate transporters in neurodegenerative diseases and potential opportunities for intervention. *Neurochem Int* 51:333–355.
- Slotboom DJ, Konings WN, Lolkema JS (2001). The structure of glutamate transporters shows channel-like features. *FEBS Lett* 492:183–186.
- Spielmann A, Gu QB, Ma CH, Huang CG, Xuan LJ, Schwarz W (2008). Inhibition of P2X7 receptor by extracts of Chinese medicine. *J Acupunct Tuina Sci* 6:286–288.
- Wadiche JI, Amara SG, Kavanaugh MP (1995). Ion fluxes associated with excitatory amino acid transport. *Neuron* 15:721–728.
- Wang HZ, Chen YG, Fan CS (1998). Review of studies on chemical constituents and pharmacology of genus *Acorus* in China. *Acta Bot Yunnan Suppl.* X pp. 96–100.

- Wang L, Ding GH, Gu QB, Schwarz W (2010). Single-channel properties of a stretch-sensitive chloride channel in the human mast cell line HMC1. *Eur Biophys J* 39:757–767.
- Wang Y, Cui JM, Ma SL, Liu YY, Yin LM, Yang YQ (2009b). Proteomics analysis of component in serum with anti-asthma activity derived from rats treated by acupuncture. *J Acupunct Tuina Sci* 7:326–331.
- Xia P, Pei G, Schwarz W (2006). Regulation of the glutamate transporter EAAC1 by expression and activation of  $\delta$ -opioid receptor. *Eur J Neurosci* 24:87–93.
- Yang LB, Li SL, Huang YZ, Wang YH, Liang JM, Zhang SQ (2006). Effect of *Acorus gramineus* and its active component  $\alpha$ -asarone on apoptosis of hippocampal neurons in epileptic young rats. *Chin Trad Herb Drugs* 37:1196–1199.
- Zerangue N, Kavanaugh MP (1996). Flux coupling in a neuronal glutamate transporter. *Nature* 383:634–637.
- Zhang D, Ding G, Shen X, Yao W, Zhang Z, Zhang Y, Gu Q (2008). Role of mast cells in acupuncture effects: A pilot study. *Explore* 4:170–177.
- Zhang D, Spielmann A, Wang L, Ding G, Huang F, Gu Q, Schwarz W (2012). Mast-cell degranulation induced by physical stimuli involves the activation of transient-receptor-potential channel TRPV2. *Physiol Res* 61:113–124.
- Zhang Y-Q, Ji G-C, Wu G-C, Zhao Z-Q (2002). Excitatory amino acid receptor antagonists and electroacupuncture synergetically inhibit carrageenan-induced behavioral hyperalgesia and spinal fos expression in rats. *Pain* 99:525–535.



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