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### **Report on practice in Umea University**

This practical training was held in collaboration with Staffan Johansson and Michael Druzin at department of Integrative Medical Biology, Umea University, Umea, Sweden, 1/03/2011-27/05/2011.

The main goals of the practice were study of patch-clamp techniques, work with dissociated brain neurons and brain slices and use of these skills to study role of TRPV channels in synaptic transmission.

Four members of the transient receptor potential vanilloid (TRPV) protein subfamily have been identified as heat-gated nonselective cation channels. They are widely expressing in mammalian's tissues, especially in CNS. It was shown that these channels are involved in thermal and pain sensation. Therefore study of the TRPV channels' role in synaptic transmission is very important for sensation mechanisms understanding.

We used dissociated single neurons with adhering synaptic terminals from medial preoptic nucleus (MPN). Whole-cell currents were measured under voltage-clamp conditions using the amphotericin B perforated-patch technique. GABA-mediated miniature inhibitory postsynaptic currents (mIPSCs) were recorded and their frequency was analyzed.

2-Aminoethoxydiphenyl borate (2-APB) was firstly introduced as an inhibitor of inositol 1,4,5-trisphosphate receptors (IP<sub>3</sub>Rs). Later It was also demonstrated capacity to block store-operated calcium entry (SOCE). But now this drug is used as an agonist of TRPV1, 2 and 3. Application of 2-APB (50 μM) increased mIPSCs frequency. The study showed that IP<sub>3</sub>-receptor antagonist xestospongin C and ryanodine-receptor antagonist ryanodine didn't prevent increasing of mIPSCs frequency showing that intracellular calcium store is not involve in 2-APB effect.

Reducing calcium concentration in extracellular solution (replacing by cobalt and buffering by EGTA) decreased the potentiating effect of 2-APB.

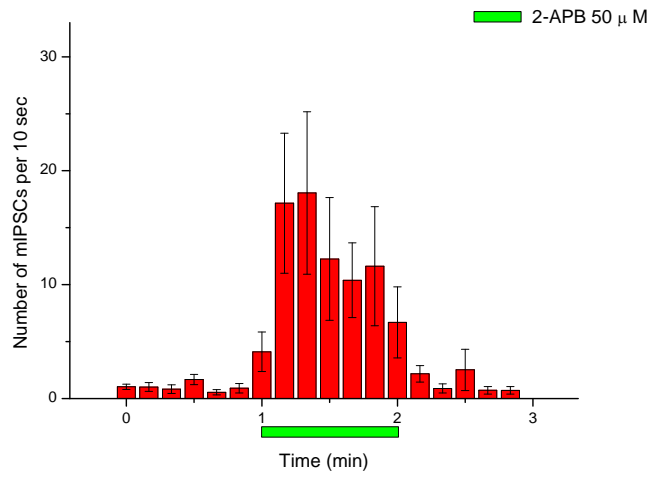
Removing of sodium ion from extracellular solution demonstrated the same effect. Therefore 2-APB induces sodium influx into the terminal that depolarizes membrane. This depolarization opens voltage-dependent calcium channels, calcium influx through these channels causes increasing of spontaneous GABA release.

The next step of this study was appreciate the role of TRPV channels in 2-APB effect. We used 100 microM 2, 2-diphenyltetrahydrofuran (DPTHF), an antagonist of TRP channels of V1, V2 and V3 types. It was shown that DPTHF totally prevents 2-APB caused enhancing of spontaneous GABA release. Also we used diphenylboronic anhydride (DPBA), an agonist of TRPV1, TRPV2 and TRPV3, which caused an increase of mIPSC frequency similar to the effect of 2-APB. So we can conclude, that increasing of mIPSCs frequency by 2-APB is caused by activating of TRPV1, 2 and 3 channels. But what type of TRPV channels is involved in this process? The using of specific antagonists of each subtype of TRPV channels will be the next step of this investigation.

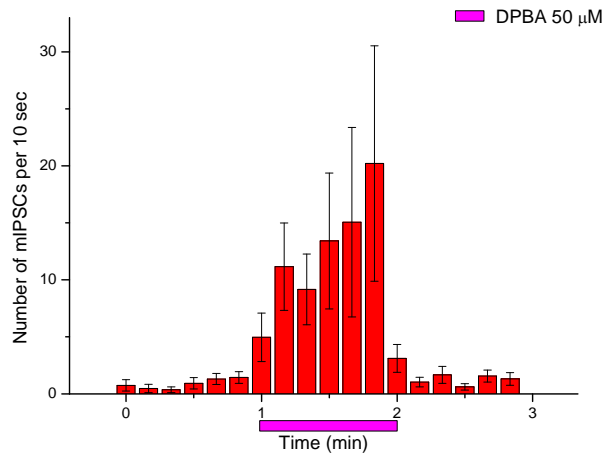
From the other hand it was important to understand which type of voltage-dependent calcium channels is involved in 2-APB action. We got preliminary data showing that 2-APB effect is mainly mediated by N-type of calcium channels.

As a result of this practice we got new data, all collected data are fully analyzed and ready for publishing. Some of data are presented in this report (see below). The practice, travel and stay were sponsored by BioN (EC Tempus grant).

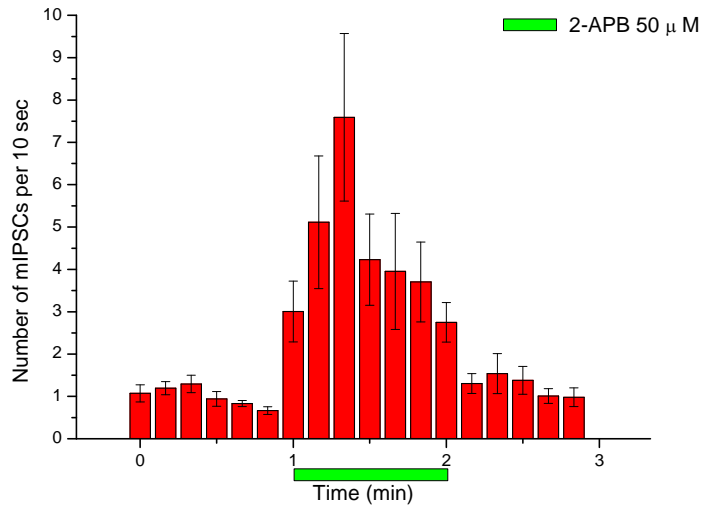
# 2-APB



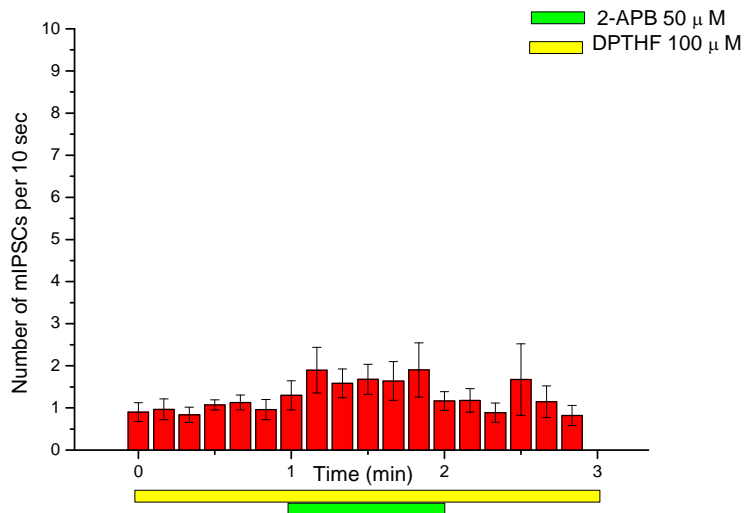
# DPBA



# 2-APB



# DPTHF+2-APB



# 2-APB after washout

