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STW 5 (Iberogast®) and its individual herbal components modulate intestinal electrophysiology of mice

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Abstract

STW 5 (Iberogast®), a phytomedicine consisting of a fixed combination of nine individual plant extracts, is widely used in the treatment of dyspepsia and motility related disorders. Little if anything is known on the possible influence on electrophysiological properties of intestinal smooth muscle by which STW 5 causes its beneficial effects. The aim of the present study was to investigate whether herbal extracts influence electrophysiological parameters of large and small intestine. For this purpose intracellular recordings of smooth muscle cell (SMC) of the circular muscle layer of different parts of mouse intestine were performed using standard microelectrode techniques. The resting membrane potential (RMP), excitatory and inhibitory neurotransmission in proximal colon, the frequency and the amplitude of slow waves in small intestine were investigated. The RMP of SMC was -46.4 ± 3.8 mV, $n = 11$ in the colon and -59 ± 1.3 mV, $n = 15$ in small intestine. STW 5 significantly depolarized the RMP of colonic (16.6 ± 2.2 mV, $n = 6$, $p < 0.05$) and jejunal (9.6 ± 1.6 mV, $n = 7$, $p < 0.05$) SMC. This depolarizing effect can be mainly attributed to the constituents of chamomile flower, Angelica root and greater celadine herb. Following the electrical field stimulations (EFSs), junction potentials are influenced in a distinct manner. Excitatory junctions potentials (EJPs) of the colon were not significantly reduced (13.1 ± 4.8 vs. 10.1 ± 2.8 n.s., $n = 6$) but fast (fIJP) and slow (sIJP) inhibitory junction potentials of the murine colon are reduced significantly by STW 5 (fIJP: 21.6 ± 8.1 vs. 11.6 ± 2.1 and sIJP: 12.1 ± 3.3 vs. 6.1 ± 1.3 $n = 6$, $p < 0.05$). The basal frequency of small intestinal slow waves was $39.5 \pm 1.4\ \text{min}^{-1}$ and the amplitude was 23.1 ± 0.9 mV, $n = 15$. STW 5 significantly reduced amplitude and frequency of the slow waves (11.7 ± 0.8 mV; $33.5 \pm 3.4\ \text{min}^{-1}$, $n = 6$, $p < 0.05$). This effect on slow waves represents the summation of effects of the nine individual phytoextracts. Whereas Angelica root and chamomile flower completely blocked the slow wave activity, bitter candy tuft increased the frequency and amplitude, greater celadine herb reduced frequency and amplitude of the slow wave, peppermint leaf reduced frequency and left amplitude unchanged and liquorice root, caraway fruit and lemon balm leaf had no effects in basic electrophysiological properties of SMC. This study demonstrates that STW 5 causes changes in SMC RMP, excitatory and inhibitory neurotransmission and slow wave rhythmicity. These effects

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represent a summation effect of different constituents of this phytotherapeuticum and prove that STW 5 has characteristic effects on intestinal electrophysiology.
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Keywords: Herbal extracts; STW 5; *Iberis amara*; RMP; Slow wave; Neurotransmission; Intestinal electrophysiology

Introduction

The pathophysiological concepts concerning functional disorders of the intestine among others include disturbances in motility and/or acid production as underlying mechanisms. Hypersensitivity of the gut and the damage of autonomic nervous system were suggested as possible additional mechanism underlying some of the functional disorders. This means that minimal disturbances of physiological processes may cause exaggerated sensations within the gastrointestinal tract (Houghton, 1999). Functional gastrointestinal disorders like functional dyspepsia and motility associated disorders like irritable bowel syndrome (IBS) or slow transit are often symptomatically treated employing herbal extracts. Among these, STW 5 is a fixed combination product which is widely used in Europe (Saller and Reichling, 2002; Gundermann et al., 2003; Rösch et al., 2006).

STW 5 is a combination of nine different herbal constituents. The individual herbal extracts are from: bitter candy tuft (15%), Angelica root (10%), caraway fruit (10%), milk thistle fruit (10%), greater celadine herb (10%), liquorice root (10%), chamomile flower (20%), lemon balm leaf (10%) and peppermint leaf (5%) with a final ethanol concentration of 31% (Reichling and Saller, 2002).

Motility patterns of the gastrointestinal tract are regulated by a number of circuits including the peristaltic reflex, migrating motor complexes, excitatory and inhibitory neurotransmission within myenteric plexus and a slow wave rhythmicity generated by the interstitial cells of Cajal (ICC). Among these, slow waves, generated by the ICC, are the electrophysiological basis for peristaltic smooth muscular contractions (Huizinga et al., 1995; Sanders, 1996). Action potentials initiated at the top of the slow waves in smooth muscle appear to propagate through the muscle layer of intestine and are often the first step in the excitation coupling mechanism leading to contraction (Hara et al., 1986; Lammers and Slack, 2001; Lammers et al., 2002; Sancholuz et al., 1975). The smooth muscle cell (SMC) itself appears to act chiefly as recipient of the slow wave, which they in turn transform into patterns of contraction (Lee et al., 1999; Stevens et al., 2000). Postjunctional mechanisms responsible for receiving and transducing neurotransmitter signals are specifically expressed by ICC. The large extracellular spaces typical between nerve terminals and SMCs may not allow effective concentrations of neurotransmitters to reach receptors expressed by SMCs. This indicates an important role for ICC in enteric neurotransmission (Ward and Sanders, 2001). The coupling of ICC and SMCs is strongly believed to be a coupling via gap-junctions. The morphological evidence for this motion has been brought up by electron microscopy but due to the lack of highly selective gap-junction blockers final functional evidence is still missing (Daniel and Wang, 1999; Daniel et al., 2001; Jimenez et al., 1999; Schultz et al., 2003). Though membrane potentials and slow wave activity can be modulated by the known excitatory and inhibitory neurotransmitters, none of the established neurotransmitter can reversibly abolish slow wave (Hara et al., 1986; Kim et al., 2003; Sanders et al., 2004).

To date little if anything is known on the possible effects of different herbal extracts or herbal combinations preparations like STW 5 on intestinal motility patterns and intestinal electrophysiology, though they are used for decades with symptomatic benefit in patients with functional dyspepsia and IBS. Therefore, the aim of this investigation was to evaluate possible effects of different single component of herbal preparations on small intestine by recording membrane potentials of circular SMCs and describes changes of resting membrane potentials (RMPs) and intestinal slow wave rhythmicity.

Materials and methods

Tissue preparation for electrophysiological experiments

Eight-week- to 10-week-old male Balb/C mice were anaesthetized by sodium pentobarbital and sacrificed by cervical dislocation in accordance with the recommendations of the government of the state Bavaria, Germany. The proximal colon and complete small bowel was removed through middle incision and placed into oxygenated (95% O_2–5% CO_2) Krebs solution of the following composition (mM): NaCl 120.35; KCl 5.9; $MgCl_2$, 2.5; NaH_2PO_4 1.2; $NaHCO_3$ 15.5; $CaCl_2$ 2.5; glucose 11.5, pH 7.4. The segment of the gut was opened along the mesenteric border, washed off from

remaining faecal material and pinned out in a sylgard-lined (Dow Corning Corp.; Midland, MI, USA) dissecting dish containing oxygenated Krebs solution. Ten millimetres wide segment of proximal colon with whole circumference or a proximal part of small intestine was separated and the mucosa and submucosa were removed, resulting in sheets of tissue consisting of circular and longitudinal muscle layers, with the attached myenteric plexus.

Intracellular electrical recording

Intracellular electrical recordings were performed as described previously (Sibaev et al., 2003; Storr et al., 2004). Sheets of muscle were pinned using approximately 150–200 micropins (15–25 μm in thickness), obtained from wolfram wire, to the sylgard-based electrophysiological chamber, with the circular muscle layer uppermost. The chamber was constantly perfused (5 ml min^{-1}; Kwik Pump, World Precision Instruments; Sarasota, FL, USA) with prewarmed (37 °C) oxygenated Krebs solution. Tissues were allowed to equilibrate for 90–120 min before the experiments were started. Capillary glass microelectrodes (borosilicate glass capillaries, 1.0 mm outer diameter × 0.58 mm inner diameter, Clark Electromedical Instruments; Edenbridge, UK) were made using a microelectrode puller (Model P-97, 3 mm wide filament, Sutter Instruments; Novato, CA, USA), filled with KCl (3 M) and had resistances in the range 80–120 MΩ. After the equilibration period, a circular SMC was impaled and the membrane potential was recorded against a ''ground'' Ag–AgCl electrode placed in the bath medium. Then the tested drugs were added in the perfusate of the organ bath in increasing concentrations. Electrical events were amplified (DUO 733 microelectrode amplifier, World Precision Instruments; Sarasota, FL, USA) and digitalized with an analogue-to-digital converter (SCB 68 interface, National Instruments; Austin, TX, USA). Permanent recordings of membrane potentials were made on a personal computer running the LABVIEW 5.0 program (National Instruments; Austin, TX, USA).

Intracellular recording of influence stimulated neurotransmission

Neurons of myenteric plexus were stimulated (15 V; 0.3 ms duration; single pulses) via platinum electrodes arranged perpendicularly to the circular muscle layer and were connected to a Grass S11 stimulator via a stimulus isolation unit (Grass SIU59; Grass Instruments, Quincy, Massachusetts, USA). Electrical field stimulation (EFS) of intrinsic neurons elicited excitatory (EJP) and inhibitory (IJP) junction potentials that contained a fast transient fIJP and a slow sustained sIJP component. Nifedipine (1 μM) was present throughout all experiments unless otherwise stated to prevent mechanical artefacts.

Data presentation and statistical analysis

The amplitudes of the junction potentials were measured in millivolts (mV) compared to the RMP before application of the electrical stimulus. RMPs (mV) and slow wave activity (frequency/amplitude) were recorded. The amplitudes were measured in millivolts (mV) as compared to the RMP. All data are given as mean plus or minus standard error (mean ± S.E.M.); n indicates the number of independent observations in intracellular recordings from different animals. Statistical differences between the treatment groups were tested using a commercial statistical package (SigmaStat, Jandel Scientific; San Rafael, CA, USA) using paired or unpaired Student's t-test where appropriate followed by Bonferroni post hoc testing. A probability of less than 5% that the null hypothesis is false was considered to be significant and a p value is given.

Drugs

The drugs used were: atropine, guanethidine, nifedipine, TTX (tetrodotoxin), ethanol (all: Sigma-Aldrich; Taufkirchen, Germany); herbal preparations: STW 5 (Iberogast®), STW 7 (STW 5 without *Iberis amara* extract); herbal extracts: *I. amara*, Angelicae radix, Carvi fructus, Silybi mariani fructus, Chelidonii herba, Liquiritae radix, Matricariae flos, Melissae folium, Mentae piperitae folium (all: Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany). The herbal extracts were either used as lyophilized extracts or dissolved in ethanol, as indicated in the methods and results section. The drugs were freshly dissolved in saline on the day of the experiment and further diluted in Krebs solution to organ bath concentrations. Drugs were added to the perfusate, and experiments were controlled for the effects of the drug solvents (ethanol).

Results

Influence of STW 5 on excitatory and inhibitory neurotransmission in colonic SMC

In the absence of drugs, circular SMC of the murine proximal colon displays a stable RMP of −46.4 ± 3.8 mV ($n = 11$). STW 5 (ethanolic or lyophilized) significantly depolarized the RMP of colonic SMC in a concentration-dependent manner ($n = 6$) (Tables 1 and 3). EFS elicited TTX-sensitive neuronal induced

Table 1. Influence of STW 5 (ethanolic solution) on RMP of circular smooth muscle of murine proximal colon

STW 5 ethanolic	Depolarization in mV
1:1000	No change
1:100	8.5±3.1*
1:50	16.6±2.2*

* = $p<0.05$.

Table 2. Influence of STW 5 (ethanolic solution) on amplitude of excitatory (EJP) and inhibitory junction potentials (fIJP and sIJP) as response to local EFS in circular smooth muscle of murine proximal colon

STW 5 ethanolic	EJP (mV)	fIJP (mV)	sIJP (mV)
Control	13.1±4.8	21.6±8.1	12.1±3.3
1:1000	No change	No change	No change
1:100	13.5±3.7	21.1±5.6	9.0±2.9
1:50	10.1±2.8	11.6±2.1*	6.1±1.3*

* = $p<0.05$.

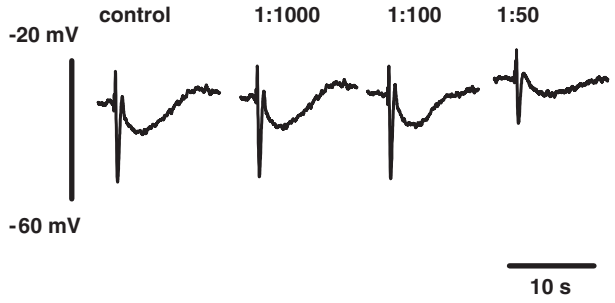

Fig. 1. Influence of STW 5 (ethanolic solution) on amplitude of excitatory (EJP) and inhibitory junction potentials (fIJP and sIJP) as response to local EFS in circular smooth muscle of murine proximal colon.

EJP (atropine-sensitive) (Storr et al., 2004) and IJP (apamin-sensitive fIJP and LNNA sensitive sIJP) (Sibaev et al., 2003) which are influenced in a distinct manner in presence of STW 5. The initial cholinergic EJP was not significantly changed, neither by ethanolic nor by lyophilized STW 5 ($n = 6$). fIJP and sIJP, as the main parameters for inhibitory neurotransmission, are significantly modified in the presence of STW 5 (ethanolic or lyophilized) in a concentration-dependent manner ($n = 6$) (Figs. 1 and 2, Tables 2 and 4). The influences of ethanolic or lyophilized STW 5 are not significantly different (Fig. 2, Tables 3 and 4), proving that ethanol leaves the reported parameters unchanged. Ethanol, when given alone in similar concentrations, did not influence EJP, fIJP or sIJP.

Table 3. Influence of STW 5 (lyophilized) on RMP of circular smooth muscle of murine proximal colon

STW 5 lyophilized	Depolarization in mV
1:1000	No change
1:100	7.9±4.1*
1:50	13.2±3.2*

* = $p<0.05$.

Table 4. Influence of STW 5 (lyophilized) on amplitude of excitatory (EJP) and inhibitory junction potentials (fIJP and sIJP) as response to local EFS in circular smooth muscle of murine proximal colon

STW 5 lyophilized	EJP (mV)	fIJP (mV)	sIJP (mV)
Control	13.1±4.8	21.6±8.1	12.1±3.3
1:1000	No change	No change	No change
1:100	14.7±3.4	22.6±7.1	10.2±2.9
1:50	13.2±2.9	13.5±5.1*	6.1±1.4*

* = $p<0.05$.

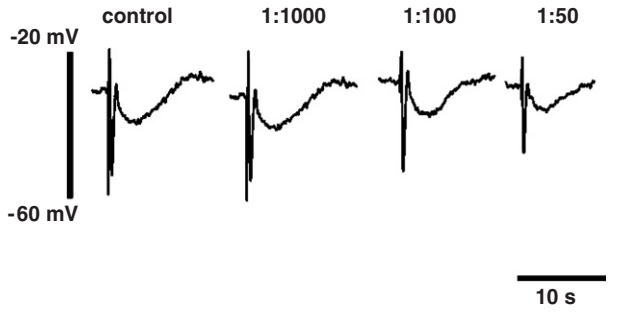

Fig. 2. Influence of STW 5 (lyophilized) on amplitude of excitatory (EJP) and inhibitory junction potentials (fIJP and sIJP) as response to local EFS in circular smooth muscle of murine proximal colon.

Recording of circular smooth muscle membrane potential and slow wave rhythmicity

In the absence of drugs, circular SMCs of the small intestine displayed a stable RMP −59±1.3 mV and a stable slow wave activity with an amplitude of 23.1±0.9 mV and a frequency of 39.5±1.4 min^{-1} ($n = 15$). Additionally, a regular spiking activity at the plate of slow wave could be recorded. The blocker of large conductance Ca^{2+}-channels nifedipine (1 μM) did not significantly alter RMP or slow wave activity, whereas the spiking activity following the slow wave depolarization was abolished. Neither the neuronal blocker TTX (3 μM) nor atropine (1 μM) or guanethidine (1 μM) had an influence on RMP, slow wave rhythmicity or amplitude and spiking activity (data not shown).

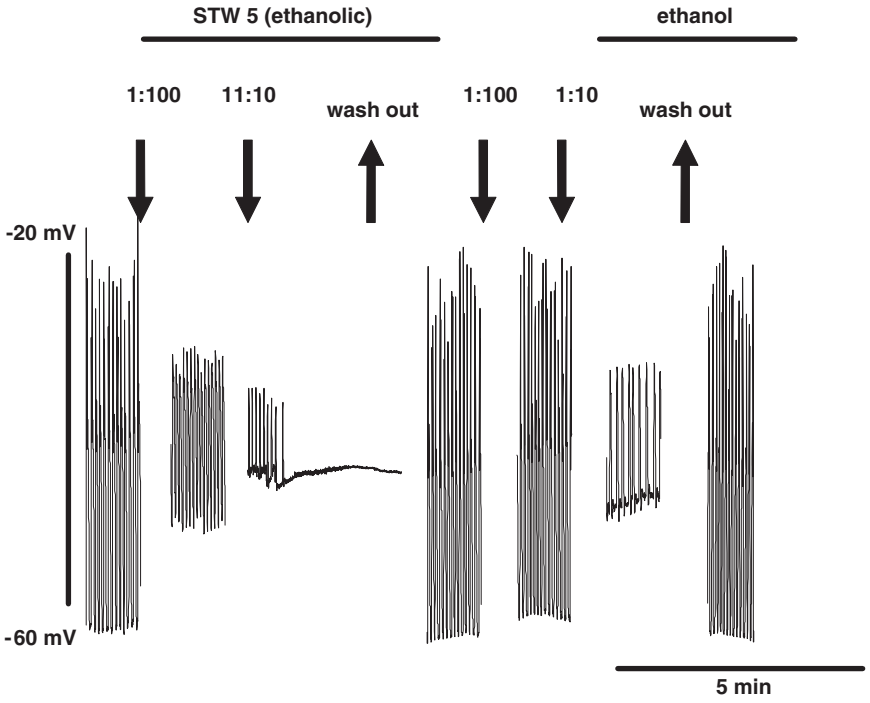

Fig. 3. Left: Inhibitory effect of STW 5 on spiking activity in response to slow waves for the 1:100 concentration and the depressant effect on slow wave activity up to abolition for the 1:10 concentration of STW 5. Right: Recordings of the same patterns in presence of pure ethanol solution in concentrations equally to the STW 5 solutions used. Please note that equal ethanol concentrations do not change the spiking activity at 1:100 and that at 1:10 ethanol does influence RMP and slow wave activity but the effects are far less than the ones observed for STW 5.

Influence of STW 5 on RMP and slow wave activity of small intestine

STW 5 used as ethanolic solution dose-dependently depolarized RMP (1:1000: $+1.5\pm1.2$ mV; 1:100: $+3.6\pm0.7$ mV; 1:50: $+6.8\pm0.6$ mV*; 1:10: $+17.0\pm0.4$ mV*; $n=7$, $*=p<0.05$) and reduced slow wave frequency (% of control: 1:1000: 109.0 ± 2.5; 1:100: 95.6 ± 2.8; 1:50: 57.9 ± 5.1*#; 1:10: abolished; $n=7$, $*=p<0.05$; $=p<0.05$ vs. ethanol-effect) and slow wave amplitude (% of control:1:1000: 117.3 ± 2.8; 1:100: 80.5 ± 13.1*; 1:50: 27.3 ± 11.5*#; 1:10: abolished; $n=7$, $*=p<0.05$; $=p<0.05$ vs. ethanol-effect) up to abolition (Fig. 3). The abolition was immediately reversible, when STW 5 was removed from the organ bath (Fig. 3). The spiking activity following slow waves was abolished at concentrations higher than 1:100 (Figs. 3 and 4).

STW 5 used as solution from lyophilized extracts dose-dependently depolarized RMP (1:1000: $+4.5\pm1.2$ mV; 1:100: $+6.6\pm1.8$ mV; 1:50: $+9.6\pm1.6$ mV*; $n=6$, $*=p<0.05$) and reduced slow wave frequency (% of control 1:1000: 116.4 ± 12.4; 1:100: 99.8 ± 7.9; 1:50: 83.3 ± 7.5*; $n=6$, $*=p<0.05$) and slow wave amplitude (% of control 1:1000: 98.9 ± 7.5; 1:100: 80.5 ± 8.5*; 1:50: 48.4 ± 10.0*; $n=6$, $*=p<0.05$) up to abolition (Fig. 4). The abolition was reversible when

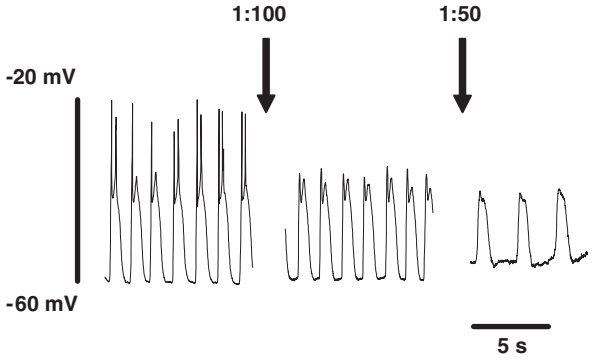

Fig. 4. The inhibitory effect of STW 5 used as lyophilized preparation on spiking activity for the concentration 1:100 and the depressant effect on slow wave amplitude and frequency following the concentration 1:50.

STW 5 was removed from the organ bath perfusate. The spiking activity following slow waves was abolished at concentrations higher than 1:100 (Fig. 4).

Influence of ethanol on RMP and slow wave activity (solvent control)

Ethanol dose-dependently depolarized RMP (1:100: no change; 1:50: $+4.0\pm0.4$ mV; 1:10: $+12.5\pm0.6$ mV*;

$n = 6$, $* = p < 0.05$) and reduced slow wave frequency (% of control 1:100: 116.7 ± 6.3; 1:50: 93.1 ± 5.1; 1:10: 82.6 ± 5.2*; $n = 6$, $* = p < 0.05$) and slow wave amplitude (% of control 1:100: no change; 1:50: 71.7 ± 13.3*; 1:10: 82.6 ± 12.8*; $n = 6$, $* = p < 0.05$). The effect was immediately reversible, when ethanol was removed from the organ bath. However, the effects of ethanol were significantly lower than those of the ethanol diluted herbal extract STW 5.

Influence of the individual herbal extracts on RMP and slow wave activity

The individual herbal extracts used in the concentration within the herbal preparation STW 5 are still mixtures of various substances. They all had individual or no effects on RMP, slow wave activity and slow wave amplitude (Fig. 5). The values of the effects of the individual lyophilized (ethanol free) herbal extracts added 1:50 into the organ bath are given in Table 5.

Discussion

Treatment of functional dyspepsia and IBS is hampered by the lack of an accepted pathophysiological knowledge. Presently, various drugs influencing motility (e.g. spasmolytics, dopaminergics and serotoninergics) or hypersensitivity (e.g. antidepressants and serotoninergics) as well as herbal extracts are used to treat these states. They all give alleviation to the patient's symptoms. Though several different clinical studies show that herbal extracts like STW 5 are potent antidyspeptic agents (Gundermann et al., 2003; Madisch et al., 2001; Rösch et al., 2002; Saller and Reichling, 2002; Rösch et al., 2006), disappointing less is known on the potential underlying mechanisms on a possible regulation of basic gastrointestinal electrophysiological parameters.

Our data clearly show that STW 5 dose-dependently depolarized RMP of large and small intestinal SMC. No individual herbal extract caused a hyperpolarization, whereas a combination of the individual extracts caused depolarization. This depolarization influences smooth muscular excitability and since the effects of the extracts are reversible, these effects are suggested to be non-toxic.

Besides a stable RMP, electrophysiology of gastrointestinal tract is additionally characterized by a slow wave rhythmicity, on which we discuss later, and smooth muscular junction potentials in response to neuronal activation. Neuronal activation, which we apply by EFS, results in an EJP and a biphasic IJP (fIJP and sIJP).

The EJP represents cholinergic excitatory neuronal pathways (Spencer and Smith, 2001) and is not changed by STW 5. In contrast, the IJP (fIJP for peptidergic inhibitory neurotransmission (Pluja et al., 1999a, b) and sIJP for nitrergic inhibitory neurotransmission (Pluja et al., 1999a, b) is reduced by STW 5 in a concentration-dependent manner, demonstrating the deep impact of this herbal preparation on neuromuscular interaction. These changes involve both, peptidergic and nitrergic neuronal mechanisms and the changes appear for both types of solutions, indicating that for the impact on neuromuscular interaction, the ethanol content is not relevant. The reported effects further present in a concentration-dependent manner and they are fully reversible.

Though of major interest, the individual phytochemical compound or compounds, to which these effects on neurotransmission can be attributed, are not identified yet. Due to the combination of nine plant extracts, the number of presently known individual phytochemical compounds exerts 300 by far (Wegener and Wagner, 2006).

More interesting are the effects of the herbal extracts on slow wave amplitude and rhythmicity. The slow waves are known to be generated by the ICC and the maximum of slow wave frequency in turn sets the maximum frequency of contractions and determines propagation characteristics of the rhythmic contractions in the intestine (Der-Silaphet et al., 1998; Hara et al., 1986; Huizinga et al., 1991a, b; Sanders, 1996). Until now no individual non-toxic drug can reversibly abolish slow wave rhythmicity. Abolition of slow wave activity and concomitant changed neurotransmission are only reported in genetically modified mice ($W/W^{+/+}$) missing the ICC (Ward et al., 1994; Ward and Sanders, 2001). Whereas the regulatory mechanisms, responsible for slow wave generation, are still unknown, frequency seems to be dependent on extracellular Ca^{2+} content as demonstrated by voltage clamp experiments (Huizinga et al., 1991a, b) and on release of intracellularly stored Ca^{2+} (Liu et al., 1995). In small intestine, an increase of slow wave activity can be achieved by drugs sensitizing IP_3 receptors (inositol 1,4,5,-trisphosphate) involved in regulation of Ca^{2+} release from the intracellular stores (Malysz et al., 2001). Electrical coupling of ICC to SMCs is believed to be a gap-junctional coupling. Due to the missing specific drugs modulating (not blocking effects) the junctional functioning, interpretation of mechanisms modulating slow wave activity remain speculative. To our knowledge this is the first report on drugs which reversibly abolish slow wave activity. STW 5 potently and significantly reduced the slow wave amplitude and the slow wave frequency. This effect is clearly different from the effect of ethanol in equimolar concentrations compared to final concentration of the herbal mixture. The reduction of the slow wave in our study is also present when STW 5 was used in an

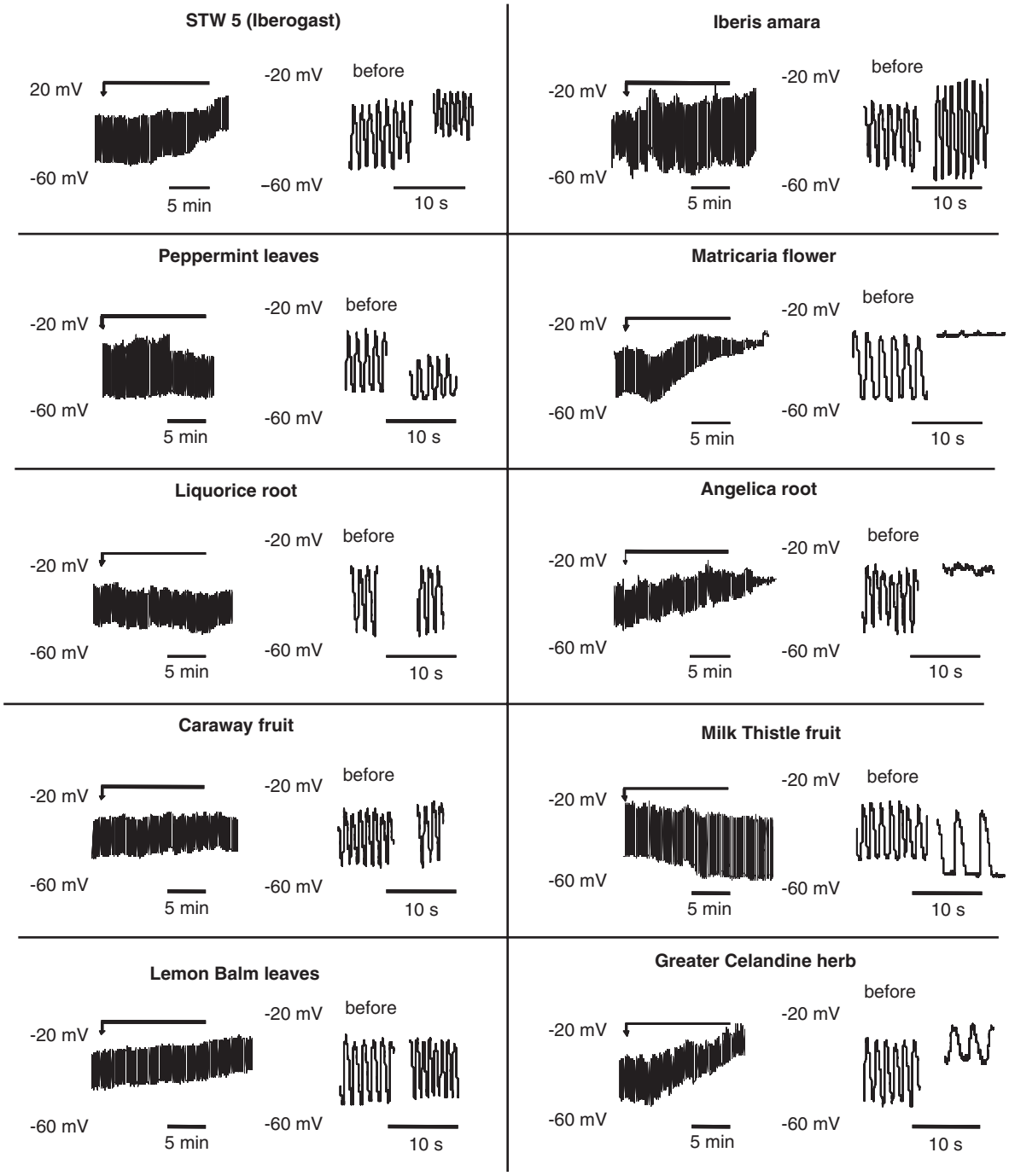

Fig. 5. Changes of slow wave activity and resting membrane potential for STW 5 and each individual phytoextracts. The phytopharmaca were used as lyophilized preparations in a concentration of 1:50.

ethanol-free lyophilized application form. As for the RMP the different individual herbal extracts had different effects on the slow wave, ranging from an increase of amplitude and frequency for *I. amara* to decrease/full blockade of amplitude and frequency for other extracts. Some of the herbal extracts like the extracts of Liquiritae radix and Carvi fructus caused no significant effect on slow wave rhythmicity. This multiple mode of action when combined in the final herbal preparation resulted in a net depressant effect on slow waves. Though STW 5 and most of the single extracts caused depressant effects on slow waves, the extract of *I. amara* augmented slow waves, again pointing on a multiple mode of action of the herbal preparation STW 5 due to the combination of different herbal extracts. This multiple mode of action might be a reason why

Table 5. Effects of STW 5 and the ethanol-free lyophilized individual extracts (bath concentration of 1:50) on resting membrane potential (RMP), slow wave frequency and amplitude

Lyophilized 1:50	RMP absolute Δ in mV	Slow wave frequency % of control	Slow wave amplitude % of control
Control	59 ± 1.3	100	100
STW 5	+9.8 ± 2.6*	83 ± 7.5*	48 ± 10.0*
Iberis amara STW 6	No change	127 ± 4.5*	131 ± 5.3*
Caraway fruit STW 5-KVI	No change	No change	No change
Milk Thistle fruit STW 5-KVII	−5.8 ± 1.6	30 ± 4.1*	113 ± 8.8
Greater celandine herb STW 5-KIX	+12.3 ± 2.4*	47 ± 11.6*	53 ± 12.0*
Angelica root STW 5-KV	12.4 ± 1.7*	Abolished	Abolished
Liquorice root STW 5-KIV	12.3 ± 2.4*	No change	No change
Peppermint leaves STW 5-KII	No change	No change	71 ± 6.9*
Matricaria flower STW 5-KIII	+19.8 ± 3.5*	Abolish	Abolish
Lemon Balm leaves STW 5-KVIII	5.2 ± 2.2	102 ± 4.5	92 ± 5.2

* = $p < 0.05$.

STW 5 is beneficial on patient's symptoms in functional disorders where more than one pathomechanism might be underlying. The multiple mode of action is further stressed by the finding that STW 5 in low concentrations shows a trend towards increasing slow wave frequency and amplitude, before at higher concentrations of same drug depressed slow wave.

Additionally to the reported effects on slow wave rhythmicity STW 5 also abolished the spiking activity of SMCs in response to slow wave rhythmically depolarization. This spiking activity following slow wave activity is the known first step in the excitation coupling mechanism leading to entrance of extracellular Ca^{2+} and contraction of smooth muscle (Lee et al., 1999; Stevens et al., 2000). In agreement to others the spiking activity in our experiments could be abolished by nifedipine, demonstrating the dependence on the functioning of Ca^{2+}-channels. Since STW 5 abolishes this spiking activity, it may be suggested that smooth muscular contractility is reduced by mechanisms influencing slow wave and mechanisms regulating smooth muscular excitability. Which individual drug components of the mixture STW 5 influences muscular excitability, most likely by blockade of large conductance Ca^{2+}-channels, has to be answered by future investigations.

Besides the effects reported here on motility parameters, extracts of Chelidonii herba and Matricariae flos show high affinity binding on 5-HT_4 receptors but functional data on the effects on gastrointestinal motility are still missing (Simmen et al., 2006). The binding at 5-HT_4 receptors gives further evidence on possible mechanisms influencing gastrointestinal motility since agonists at the 5-HT_4 receptor are accepted potent prokinetics (Cucchiara, 1996). Interestingly, high-affinity receptor binding of the extracts of Chelidonii herba on the muscarine M_3 receptor was proven. This receptor is involved in cholinergic stimulation of SMCs (Simmen et al., 2003, 2006).

Effects of STW 5 or its constituents on motility are rarely characterized. In guinea-pig gastric smooth muscle strips from fundus and corpus STW 5 potently reduced basal tone whereas the tone of antral smooth muscle strips remained unchanged. In contrast, phasic activity and amplitude of antral contractions was increased (Hohenester and Schemann, 2003; Hohenester et al., 2004; Schemann et al., 2006). This may indicate that gastric adaptative relaxation of fundus and corpus is enhanced and antral activity is increased, both desired effects in patients with dyspepsia and impaired gastric emptying. The individual constituents showed different effects on the observed parameters, suggesting that the combination of the different extracts in STW 5 is essential basis for the balanced additive effect (Schemann et al., 2006). Comparable to our findings, the individual extracts caused different effects which resulted in an additive/compensatory effect for the different herbal components of STW 5.

In summary this investigation shows that STW 5 and the individual herbal extracts modulate multiple basic parameter such as RMP, colonic inhibitory neurotransmission as well as slow wave amplitude and rhythmicity of mouse small intestinal SMC which are the electrophysiological basis of smooth muscle contractility and motility phenomena of the intestine. The effects of the individual herbal extracts used in the herbal preparation STW 5 add up to a total effect and the addition of individual effects might be the reason why STW 5 is helpful for patients with relieving symptoms of functional gastrointestinal disorders of various origins and presently unknown pathophysiologies.

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