

*In vitro studies*

## An article on the mechanism of action of ivy

*Frank Runkel, Gießen, Lars Prenner and Hanns Häberlein, Bonn / Preparations made of ivy leaves have been used as expectorants for some considerable time. In addition to their expectorant effect, the ingredients thereof also have secretolytic and spasmolytic properties. Immunohistochemical and biophysical studies have shed new light on their eventual mechanism of effect.*

The management of illnesses with herbal medicines is one of the oldest forms of treatment.

Following the introduction of the Medicines' Act in 1978, it was stipulated that each medicine first be licensed before being marketed. The main purpose of each licence is to provide proof of the pharmaceutical quality, efficacy and safety of the respective medicine. In principle, this also applies to phytopharmaceuticals, for example, ivy preparations. According to the Medicines' Act, a herbal extract constitutes the medicinal agent of a given preparation, and has to be defined via the precise description of its manufacture and analysis.

Ivy is used as an expectorant for obstructive respiratory tract conditions accompanied by productive coughing (figure 1) (1).

Therapeutically, both its secretolytic and bronchospasmolytic effects are made use of. Clinical studies with ivy leaf dry extracts (drug to

extract ratio, DER: 5 – 7.5:1) show a distinct improvement in major lung function parameters which were measured by means of spirometry and bodyplethysmography, as well as an improvement in the well-being of patients (2 – 5). In one study for example, the verum was, compared with placebo, significantly more effective, both clinically and statistically, in respect of the parameters vital capacity, residual volume and respiratory resistance (6). Apart from their efficacy, the excellent tolerability of ivy preparations has been proved in toxicological studies as well in clinical studies and practice documentation (7–11). The retrospective assessment of the treatment of more than 52.000 children with a cough syrup containing a dry extract of ivy leaves (DER 5 – 7.5:1) showed, for example, that undesirable side effects occurred in only 0.22 per cent of all cases (12). The extract is therefore an expectorant with

proven effectiveness and excellent tolerability, which is used within the context of rational phytotherapy.



Fig. 1: Preparations of ivy leaves are used as expectorant since long time



Up until now, irritation of the gastric mucous membrane coupled with reflex stimulation of the vagus nerve and subsequent excessive secretion production in the goblet

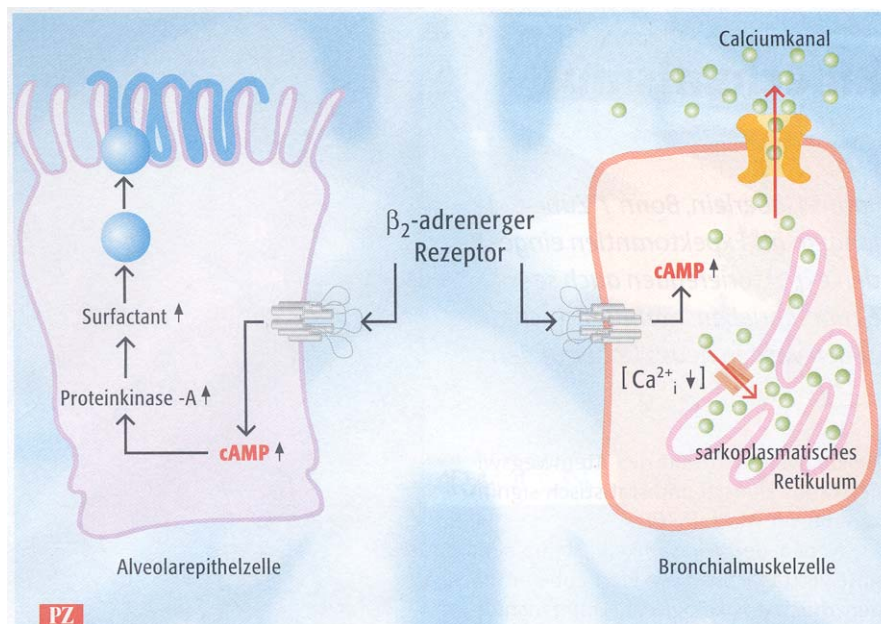


Fig. 2: Influence of the stimulation of  $\beta_2$  adrenergic receptors on the production of surfactant in alveolar type II cells (left) and on the intracellular concentration of  $Ca^{2+}$  in cells of the bronchial muscle (right) (explanations are given in the text)

liquid film of the alveolar cells, where it lowers the surface tension thereof.

Surfactant is a mixture of phospho-lipids and surfactant proteins A to D. The production thereof is initiated by the signal substance cAMP (cyclic adenosine monophosphate) which, in turn, is produced with the help of a key enzyme, adenylate cyclase. This enzyme is activated as a result of binding of the neurotransmitter adrenaline to the  $\beta_2$ -adrenergic receptor (fig. 2, left) (14).

$\beta_2$ -adrenergic receptors are also found on the cells of non-striated bronchial muscles. The stimulation thereof also increases the cAMP concentration, which results in a drop in the intracellular concentration of calcium as a consequence of elevated  $Ca^{2+}$  accumulation in the sarcoplasmic reticulum and  $Ca^{2+}$  outflow through calcium channels (figure 2, right). As a result, myosinkinase is phosphorylated to a greater degree by cAMP-dependent protein kinase (PKA) and thus becomes less active: this leads to relaxation of the bronchial muscles.

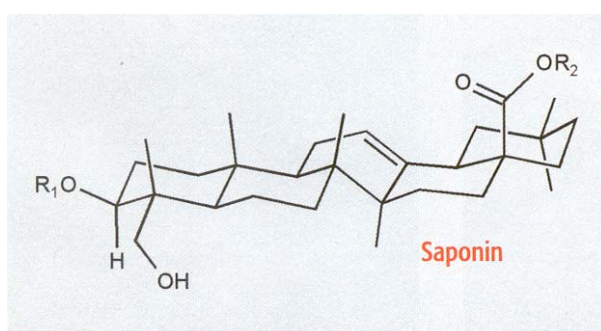
### Regulation of signal transduction

The  $\beta_2$ -adrenergic receptor ( $\beta_2$ -R) belongs to the family of G-coupled protein receptors (GPCR), is characterised structurally by seven transmembrane domains and is situated within the cell membrane in so-called functional microdomains (lipid rafts, caveolae). Following the binding of adrenaline to the  $\beta_2$  receptor, the G protein complex, consisting of the subunits  $\alpha$ ,  $\beta$  and  $\gamma$ , moves from the cytosolic side and settles on the  $\beta_2$  receptor and adrenaline complex. After GDP (guanosine diphosphate) is exchanged for GTP (guanosine triphosphate) at the  $\alpha$  subunit, the two subunits  $\beta$  and  $\gamma$  split off and the GTP-laden  $\alpha$  subunit travels within the cell membrane until it reaches its target protein, for example,

cells in the bronchial mucus membrane was thought to constitute the mechanism of action of dry ivy leaf extract. This theory does not, however, explain the spasmolytic effect that the saponins contained in the extract have on the bronchial muscles, which, for example, was able to be demonstrated in the guinea-pig by means of the dose-dependent inhibition of bronchoconstriction induced by PAF (platelet activating factor) (13). It can be concluded from this finding that the extract possesses a  $\beta_2$ -mimetic effect, which would plausibly explain the secretolytic and bronchospasmodic effects thereof.

### $\beta_2$ receptors in the lung

Gas exchange, i.e. the uptake of oxygen and the release of carbon dioxide, takes place in the air cells (alveoli) situated in the lungs. Compared with aqueous boundary layers, the liquid film of the alveoli has a significantly lower surface tension, thereby allowing for optimum ventilation of the lungs, easier gas exchange and the better transport of mucus. The surface factor surfactant plays a leading role thereby. It is produced constantly in type II alveolar cells, packed in small containers (lamellar bodies) and transferred to the surface by exocytosis. During exocytosis, the biomembranes of the lamellar bodies merge with the cell membrane, as a result of which surfactant is transferred to the



	$R_1$	$R_2$
Hederagenin	H -	H -
$\alpha$ -Hederin	L-Rhamnose-L-Arabinose -	H -
Hederacosid C	L-Rhamnose-L-Arabinose -	L-Rhamnose-L-Glucose-L-Glucose -

Fig 3: Main saponins in the dry extract of ivy

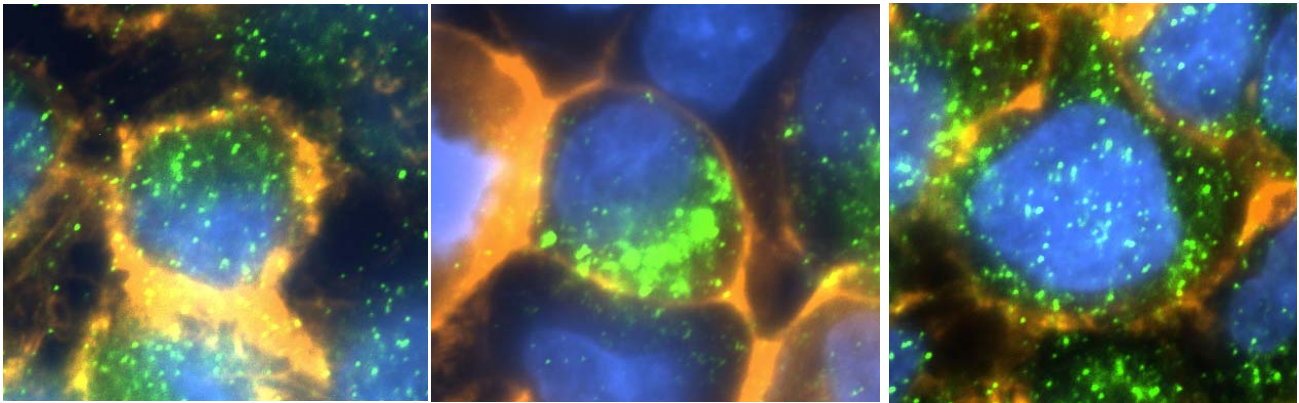


Fig. 4: Immunohistochemical detection of  $\beta_2$  receptors (green fluorescent). A) untreated A 549-cell, B) internalization of  $\beta_2$  receptors after stimulation with 10 $\mu$ M terbutalin and C) Inhibition of  $\beta_2$  receptor internalization within stimulating conditions (10 $\mu$ M terbutalin) resulting from preincubation with 1 $\mu$ M  $\alpha$ -hederin

adenylate cyclase (AC). The GTP is used here to activate AC, which now, for its part, synthesises the secondary messenger cAMP.

The  $\beta_2$  receptor-ligand complex is phosphorylated by phosphokinases such as phosphokinase A (PKA) or  $\beta$ -adrenergic receptor kinase 2 ( $\beta$ ARK or GRK<sub>2</sub>) and desensitised by means of cytoskeletal component binding such as the protein arrestin. The further binding of cytosolic proteins such as AP-2 (associated protein 2) and clathrin leads to the accumulation and trapping of  $\beta_2$  receptor-ligand complexes and the formation of coated pits ("invaginations") in the cell membrane.

If signal transduction exceeds a certain intensity, the  $\beta_2$  receptor-adrenaline complex is absorbed (internalised) into the cell by means of endocytosis, after accumulating in the coated pits, in the form of early endosomes. In this way, the number of membrane-based receptors is reduced and an overshooting of the signal prevented. When the signal is strong, because of a constant high extracellular ligand concentration for example, the internalised receptor is broken down in the cytoplasm. With the help of CURL (compartment of uncoupling of receptor and ligand), it can, however, be recycled and incorporated in the cell membrane so as to

strengthen the extracellular signal after a drop in the ligand concentration. In this way, optimum signal transduction, which is necessary for the functionality of the cell, is ensured (15, 16).

► This means: activation of the  $\beta_2$  receptor triggers a signal cascade before the receptor is internalised. If the receptor remains in the cell membrane, the messenger adrenaline is able to dissociate and the receptor is able to be reactivated.

#### Ivy extract saponins

The major components in ivy leaf extracts are oleanolic saponins, which are essentially represented by the aglucone hederagenin, the monodesmoside  $\alpha$ -hederin and the bisdesmoside hederacoside C (fig. 3). The extract is standardised to hederacoside C. The European Pharmacopoeia Commission stipulates in the monograph a minimum content of 3.0 per cent hederacoside C pertaining to ivy leaves. In addition to the saponins already mentioned, other compounds of this substance class are contained in ivy extract. The content thereof is, however, not of note.

Receptor binding studies have not been able to demonstrate a direct  $\beta_2$ -adrenergic effect for saponins (17). An indirect  $\beta_2$ -

mimetic effect has, therefore, been postulated in respect of an answer to the secretolytic and bronchospasmodic effects of the extract.

#### $\alpha$ -hederin inhibits internalisation

The internalisation of  $\beta_2$ -adrenergic receptors was able to be demonstrated, by means of immunohistochemistry, on A549 cells (type II alveolar cells). For this purpose, the cells were first incubated with a primary antibody (mouse), which specifically recognises the  $\beta_2$  receptor. In order to render the resulting  $\beta_2$  receptor-antibody complex visible, a green, fluorescence-marked secondary antibody (goat), which was aimed specifically at the mouse antibody, was added. In this way, the  $\beta_2$  receptors were able to be clearly recognised as small green spots under the fluorescence microscope (fig. 4A).

Following the further addition of 10  $\mu$ M terbutaline, a specific  $\beta_2$  receptor agonist, intensive internalisation of the  $\beta_2$  receptor in the form of early endosomes (large green fluorescent spots) was able to be detected within as little as 20 minutes (fig. 4B). By comparison, the untreated control cells exhibited no essential internalisation of  $\beta_2$  receptors.

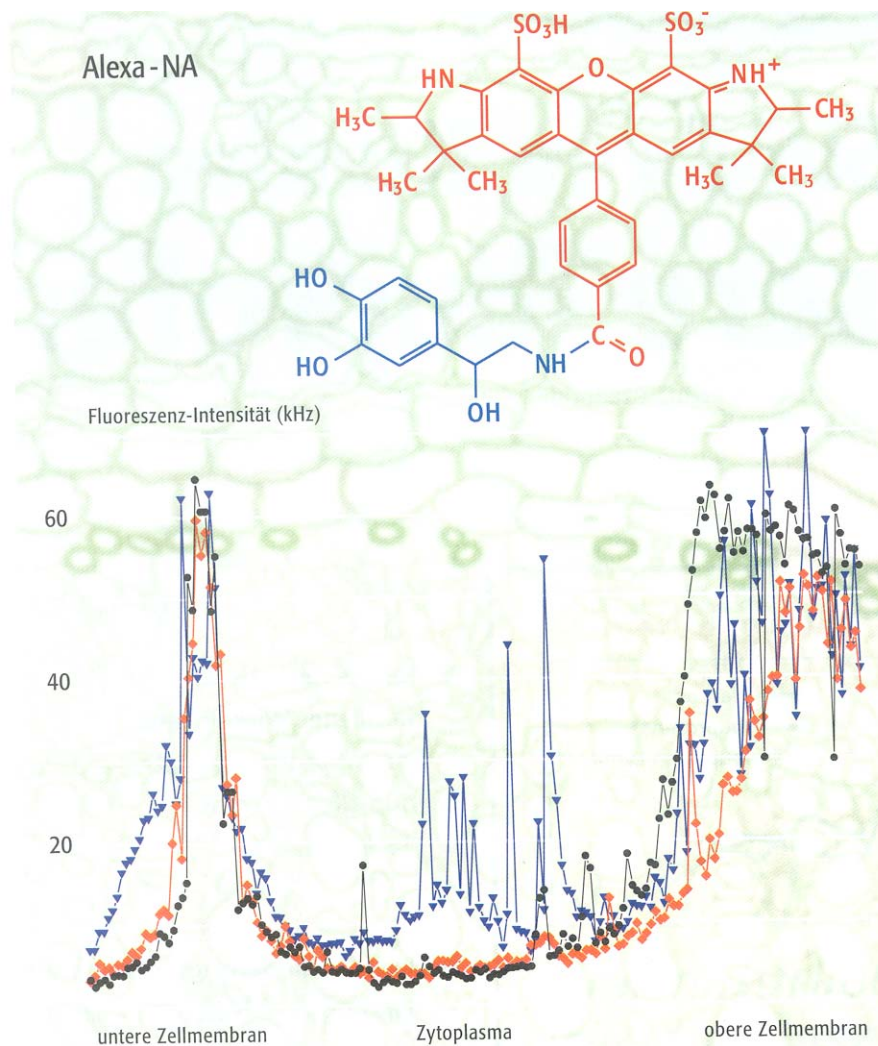
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Fig. 5: Cell scans of A549 cells after incubation with 10nM Alexa-NA (black), after additional application of 1 $\mu$ M terbutalin (blue) and after preincubation with 1 $\mu$ M  $\alpha$ -hederin and subsequent stimulation with 10nM Alexa-NA and 1 $\mu$ M terbutalin (red) (18,19)

After pre-incubation with 1  $\mu$ M hederin for a period of 24 hours, the cells were rinsed thoroughly with incubation buffer and the ivy saponin was removed. Remarkably, the immunohistochemical treatment of these cells showed, after stimulation with 10  $\mu$ M terbutaline, no mentionable internalisation of  $\beta_2$  receptors in comparison with the positive control (fig. 4C).

►  $\alpha$ -hederin therefore inhibits the intracellular uptake of  $\beta_2$  receptors under stimulating conditions and thus leads to increased  $\beta_2$ -adrenergic cell reactivity (18, 19), as adrenaline

is able to bind once again to the receptor on the cell surface.

This finding was able to be confirmed by means of the laser-aided cell scanning of living A549 cells and the recording of the resulting fluorescence profiles. For this purpose, the cells were first immobilised in monolayer form on a cover glass. The highly focussed light of an Argon laser, in the form of an ellipsoid beam, was allowed to travel axially through the cells, thereby simultaneously recording a characteristic two-peak profile, which was recognisable as a result of the autofluorescence of excitable molecules, for exam-

ple, certain proteins. Whereas the autofluorescence of the lower biomembrane led to the first peak, the cytoplasm showed a faint fluorescence trace, which led, however, into the second peak as soon as the beam hit the upper biomembrane.

For the detection of  $\beta_2$  receptors by means of cell scans, noradrenaline labelled with the fluorescent dye Alexa 532 (Alexa NA) was used. Its specific binding to  $\beta_2$  receptors and A549 cells as well as hippocampal neurons has been able to be demonstrated (18, 19). Following incubation of the labelled cells with 10 nM Alexa NA, individual peaks of internalised  $\beta_2$  receptor-ligand complexes, localised to early endosomes (figure 5), were observed after 20 minutes in the cytoplasm of the living cells. In the control test, the number of internalised  $\beta_2$  receptors was able to be increased significantly by the additional incubation of the cells with 1  $\mu$ M terbutaline.

When the cells are pre-incubated for 24 hours with 1  $\mu$ M  $\alpha$ -hederin and, subsequent to thoroughly rinsing out the saponins, treated with 10 nM Alexa TA and 1  $\mu$ M terbutaline, however, only a small number of peaks can be found in the cytoplasm after 20 minutes in contrast to the positive control. That means: no mentionable  $\beta_2$  receptor internalisation occurred, not even under highly stimulating conditions (fig. 5, red curve) (18, 19). This effect was not able to be detected with hederagenin or hederacoside C.

### Significance of results

The inhibition of  $\beta_2$  receptor internalisation through  $\alpha$ -hederin – under even highly stimulating conditions – was able to be demonstrated in vitro in alveolar type II cells by means of immunohistochemistry and using a biophysical procedure. As a result of this indirect effect on  $\beta_2$  receptor regulation, the  $\beta_2$ -adrenergic reactivity of the cells increases. The stimulation of the

receptors stimulates type II alveolar cells to produce more surfactant, which is able to liquefy viscous mucus. This secretolytic effect ultimately also calms coughing.

Under  $\beta_2$ -stimulating conditions, bronchial muscle cells lower the intracellular  $\text{Ca}^{2+}$  level. In bronchitis cases, this leads to relaxation of the cramped bronchial muscles and plausibly explains the bronchospasmolytic effect of ivy.

Remarkably, only  $\alpha$ -hederin and not hederagenin or hederacoside C was seen to inhibit  $\beta_2$  receptor internalisation. Hederacoside C can, however, be regarded as a prodrug, as it is converted in vivo, with the help of esterases (splitting away of the sugar chain bound via an ester function), into  $\alpha$ -hederin. The glycosidic bond does not split into the aglucone. Absorption studies show that hederacoside C is hardly detectable, as it is broken down into  $\alpha$ -hederin; the aglucone hederagenin, on the other hand, is not detected at all.

► The demand of 3.0 per cent minimum content for hederacoside C therefore makes sense, although the substance itself has no effect on the regulatory process of the  $\beta_2$ -adrenergic receptor.

A blood plasma concentration of 0.66  $\mu\text{M}$  was found for  $\alpha$ -hederin in initial absorption studies performed on humans. In the tests, a concentration of 0.5  $\mu\text{M}$  was sufficient to reach a roughly

60 per cent inhibition of receptor internalisation. It can be concluded from this that  $\alpha$ -hederin is sufficiently bioavailable.

The results obtained from cell cultures plausibly prove an eventual mechanism of action for ivy at molecular and cytobiological level. They therefore reinforce the importance of ivy extracts as rational phytopharmaceuticals for the treatment of obstructive respiratory tract diseases accompanied by coughing.

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