Commentary: Gabapentin-Lactam and Gamma-Aminobutyric Acid/Lactam Analogs: The Enigma of Their Mechanism of Action

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The previous article in this issue of Oral & Craniofacial Tissue Engineering, on the increase in proliferation of mesenchymal stem cells by gabapentin-lactam and other gamma-aminobutyric acid (GABA)-lactam analogs, prompted the present author to reconsider the probable mode of action of these compounds.

The first publication on the possible mechanism of action of gabapentin-lactam appeared in 2000, when Jehle et al described the reduction by this drug of the oxygen glucose deprivation–induced neuronal glutamate release from rat hippocampal slices and its neuroprotective effect on ganglion cells in a model of acute retinal ischemia. The latter model is characterized, among others, by an increase in vitreous glutamate. Then, it was assumed that gabapentin-lactam acted as an opener of plasmalemmal adenosine triphosphate (ATP)–sensitive potassium ion (K+) channels (KATP) to decrease excitotoxic extracellular glutamate and also as an opener of mitochondrial antiapoptotic ATP-sensitive K+ channels (mitoKATP). This assumption was based on the fact that the KATP channel antagonist glibenclamide potently reversed the decrease in extracellular glutamate, whereas the KATP opener minoxidil mimicked the effect of gabapentin-lactam. An additional intracellular, ie, mitochondrial, locus of action of gabapentin-lactam was surmised from the condition that the zwitterionic and hydrophilic parent drug of gabapentin-lactam, gabapentin, which is unable to penetrate cell membranes, in fact also diminished the oxygen glucose deprivation–induced neuronal glutamate release from rat hippocampal slices but was unable to mimic the neuroprotective effects of gabapentin-lactam on ganglion cells subjected to retinal ischemia. As a mitoKATP activator, gabapentin-lactam depressed the mitochondrial membrane potential. The involvement of mitoKATP in the mode of action of gabapentin-lactam was further confirmed by the use of 5-hydroxydecanoate (5-HD), which selectively antagonizes mitoKATP and also affects the mitochondrial formation of reactive oxygen species. 5-HD prevented the increase in the survival of retinal ganglion cells caused by gabapentin-lactam. The same was true for glibenclamide, which blocks both plasmalemmal and mitochondrial KATP channels. The role of gabapentin-lactam as a novel neuroprotective substance has also been demonstrated in vivo. In a transgenic mouse model of Huntington disease, gabapentin-lactam, but not gabapentin, had a beneficial effect on motor abilities. Moreover, a substantial reduction in the size and density of neuronal nuclear and cytoplasmatic inclusions was observed under gabapentin-lactam treatment. The pharmacokinetics of gabapentin-lactam yielded a mean plasma concentration near the median effective concentration of gabapentin-lactam to open mitoKATP (about 10 μmol/L). Interestingly, in vitro, but not in vivo, gabapentin is spontaneously transformed into the lactam, which raises the question of whether the reported neuroprotective effects of the parent drug, gabapentin, in animal models should in fact be attributed to gabapentin-lactam, possibly admixed as an impurity to gabapentin used in the aforementioned models.

In addition to the neuroprotective activity of gabapentin-lactam, neurotrophic effects of this compound have been described. In cultured hippocampal neurons, gabapentin-lactam, but not gabapentin, enhanced the formation of dendritic filopodia, which are necessary for synapse formation. Gabapentin-lactam also induced a network of F-actin-containing neurites. Gabapentin-lactam increased the addition, but also the elimination, of new branches. 5-HD prevented its effects on dendrite and branch formation, again pointing to a mitochondrial locus of action.

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As is known, mitochondria supply energy to the cell by synthesis of ATP. Respiring mitochondria transport hydrogen ions out of the mitochondria into the cytoplasm, thus forming both the transmembrane potential and the pH gradient over the mitochondrial membrane. Influx of K⁺ as a result of the opening of mitoKATP diminishes only the electrical gradient, not the pH gradient, over the mitochondrial membrane. Consequently, mitochondria no longer have to maintain two gradients and therefore may become more resistant to stress by saving energy. Possibly on the basis of this condition, mitoKATP are essential for cardiac and cerebral ischemic preconditioning.⁹–¹¹ This phenomenon relates to the fact that a short period of ischemia renders tissues more resistant to subsequent longer periods of ischemia. Thus, cellular protection is seen after short-term, moderate perturbation of the physiologic energy supply, ie, of the mitochondrial homeostasis. A moderate mitochondrial stress obviously primes the entry of mitochondria into a stress-resistant state and activates endogenous defense mechanisms.² Ischemic preconditioning is transmitted, among other factors, by a release of adenosine, activation of adenosine A₁ receptors, and resultant activation of mitoKATP.⁹ Correspondingly, drugs opening mitoKATP mimic preconditioning, whereas mitoKATP antagonists decrease the protective effect. In addition to adenosine, ischemic preconditioning also involves reactive oxygen species (ROS), probably occurring upstream of the preconditioning-related activation of mitoKATP.¹² Therefore, mitoKATP activation is no longer assumed to increase ROS generation but may decrease mitochondrial ROS production during reperfusion.¹² Interestingly, also, growth can be promoted by exposure to ROS, eg, when smooth muscle cells meet low levels of ROS.¹³ Especially the superoxide anion radical (O₂⁻) then acts as a growth-promoting signaling molecule, and mitochondrial ROS, being involved in the communication between nucleus and mitochondria, are second messengers in events required for differentiation.¹⁴ In this way, mitochondria codetermine cell survival.² Moreover, ROS have been shown to be necessary in molecular processes involved in signal transduction, synaptic plasticity, and memory formation.¹⁵,¹⁶

Obviously, ROS have additional functions besides those related to oxidative stress. Low, quasiphysiologic ROS concentrations are produced within the mitochondria, since 1% to 5% of the O₂ consumed during respiration is only partially reduced to O₂⁻⁻. Both mitochondrial complexes I and III release O₂⁻⁻ physiologically, ie, by slightly aberrant electron transfer, and inhibition of these complexes markedly enhances ROS production.¹⁷–¹⁹

Recently, the measurement of ROS formation in mitochondria from neocortical specimens has been established via monitoring ROS-mediated conversion of dihydrorhodamine 123 to fluorescent rhodamine 123 (Rh123).¹⁶ The increase in the concentration-response curve of the complex I inhibitor rotenone on ROS generation was much more pronounced than that of rotenone on mitochondrial [³H]-choline uptake (which indicates changes in the mitochondrial membrane potential [ΔΨₘ]). Thus, mitochondrial ROS generation was reflected by Rh123 fluorescence, although this fluorescence may also mirror changes in ΔΨₘ. To validate this approach further, oxygen has been substituted by nitrogen and thereby markedly reduced the ROS signal by ~78% in rat and by ~72% in fresh human neocortical synaptosomes. In addition, the inhibitor of electron flow at complex I, rotenone, and at complex III, antimycin A, but not other mitochondrial complex blockers, increased ROS production.

To elucidate the possible mode of action of gabapentin-lactam and the GABA-lactam analogs applied in the aforementioned study on proliferation of mesenchymal stem cells, the present author and colleagues have also tested some of these compounds in a model of mitochondrial ROS formation. Obviously, the test drugs enhanced the proliferation of mesenchymal stem cells at nanomolar concentrations. In comparison, Fig 1 shows an example of the effect of these drugs on mitochondrial ROS formation (unpublished data).

The 95% confidence interval (CI) bars at log [concentrations] from −7.5 to −6 of 4-phenyl-GABA-lactam are located above the continuous curve. Obviously, the increasing part of the (imperfectly) fitted continuous curve was too far to the right or the positive curve slope was too steep, according to the composition of an increasing and a decreasing logistic function. Therefore, the data points from log[concentrations] −7.5 to −4 were refitted with another equation. Its composition of two logistic functions that only increased, restricted to the increasing parts of the data points, reflected the obvious plateau in the nanomolar concentration range much better. The parameter estimate Emax₁ assesses this plateau; Emax₂, the second and higher maximum of the curve, follows on the right. pEC₅₀₁ and pEC₅₀₂ reflect the first and second inflection points of the two increasing logistic functions. The corresponding line shows the refitted curve (Fig 1). Table 1 displays the estimated parameters using the composition of the two only increasing logistic functions.

To determine whether mitoKATP are indeed unrelated to a primary mitochondrial ROS generation,¹² the concentration-response curve of 4-phenyl-GABA-lactam in the abscissa range of log[concentrations] −7.5 to −4 was repeated in the presence of mitoKATP antagonists glibenclamide (10 μmol/L) and 5-HD (100 μmol/L). Neither glibenclamide nor 5-HD significantly
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Influenced the concentration-response curve or the corresponding parameter estimates $E_{\text{max}1}$, $E_{\text{max}2}$, $\text{pEC}_{50-1}$, and $\text{pEC}_{50-2}$ significantly (data not shown). Obviously, already nanomolar concentrations of 4-phenyl-GABA-lactam were able to increase slightly the ROS generation, as seen from the 95% CIs of the $E_{\text{max}1}$ estimate in Table 1, which did not overlap 100%.

Evidence is presented in the report of Sauerbier et al that, for instance, 4-phenyl-GABA-lactam increased the proliferation of mesenchymal stem cells at nanomolar concentrations. Are the slight, but significant, elevations of mitochondrial ROS synthesis and the increased proliferation causally linked? Such a link indeed seems reasonable, despite the fact that mitochondria of rat brain tissue have been investigated but mesenchymal stem cells obtained from sheep have been cultured and analyzed. The assumption of a causal link will be substantiated in the following.

The mitochondrial preparations most probably produced ROS in a quasiphysiologic fashion. This means that primarily $O_2^{-}$ formation as a nonenzymatic process is dependent on oxygen concentration, since the substitution of oxygen by nitrogen markedly reduced the ROS signal (by 61%). Interestingly, mesenchymal stem cells can tolerate low oxygen levels and then differentiate into osteoblasts at higher oxygen levels. The two aspects of effects of GABA-lactam analogs such as 4-phenyl-GABA-lactam, ie, their effects at nanomolar concentrations (1) on the production of ROS in isolated mitochondria of rat brain tissue and (2) on the proliferation of sheep mesenchymal stem cells in cell culture, are probably related.

The author assumes that the slight mitochondrial elevations of ROS resulting from nanomolar concentrations of GABA-lactam analogs, such as 4-phenyl-GABA-lactam, represent the physiologic and beneficial aspects of mitochondrial ROS generation. The reason
for this assumption is the fact that the proliferation of stem cells occurred exactly in that concentration range of the tested drugs. The mitochondrial elevations of ROS at nanomolar concentrations of GABA-lactam analogs came along with a slight increase in \( \Delta \Psi_M \) as could be learned from the concentration-response relationship of mitochondrial [3H]-choline uptake (data not shown).

The authors’ group did not find evidence for an involvement of mitoK\(_{\text{ATP}} \) in ROS production since the mitoK\(_{\text{ATP}} \) blockers glibenclamide and 5-HD did not influence the concentration-response curve of GABA-lactam analogs (data not shown). However, as mentioned earlier, mitoK\(_{\text{ATP}} \) may play a role downstream of mitochondrial ROS generation.\(^{12} \)

Restrictively, it should be stated, of course, that \( \Delta \Psi_M \) was observed in brain tissue mitochondria but not in the mitochondria of the stem cells under study. Nevertheless, the parallelism between the slight elevations of ROS (and of \( \Delta \Psi_M \)) at nanomolar concentrations of only those test drugs that enhanced stem cell proliferation suggests that the events are causally linked.

REFERENCES