In vitro-evaluation of galanthamine derivatives for examination of the nicotinic acetylcholine receptor system

A.Schildan[1], M. Samochocki[2], C. Christner[2], A. Maelicke[2], F. Rösch[1]
[1]Institut für Kernchemie, [2] Institut für Physiologische Chemie und Pathobiochemie,
Johannes Gutenberg- Universität, Mainz

The galanthamine derivatives which were synthesised for examining cholinergic neurotransmission under the aspect of allosteric potentiation were 6-O-demethyl-6-O-fluoroethylgalanthamine, 10-N-demethyl-10-N-fluoroethylgalanthamine and N-methylgalanthaminium (Fig. 1, for syntheses details cf. [1]).

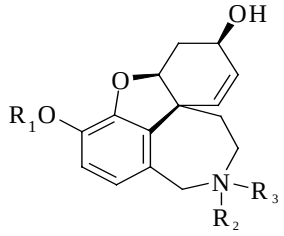

Fig.1:
Structures of galanthamine and the derivatives prepared:
galanthamine (1): R_1=CH_3, R_2=CH_3;
6-O-demethyl-6-O-fluoroethylgalanthamine (2):
R_1=CH_2CH_2F, R_2=CH_3;
10-N-demethyl-10-N-fluoroethylgalanthamine (3):
R_1=CH_3, R_2=CH_2CH_2F;
N-methylgalanthaminium (4): R_1=R_2=R_3=CH_3

In vitro evaluation was performed using the fura-2-calcium imaging method and whole-cell measurements on HEK-293 cells stably transfected with human α4β2 nAChR. The latter method showed 2 and 3 to have only a weak allosteric potentiating effect. The increased nicotinic response for those compounds was only 50% of that observed in the case of 1 (Fig.2).

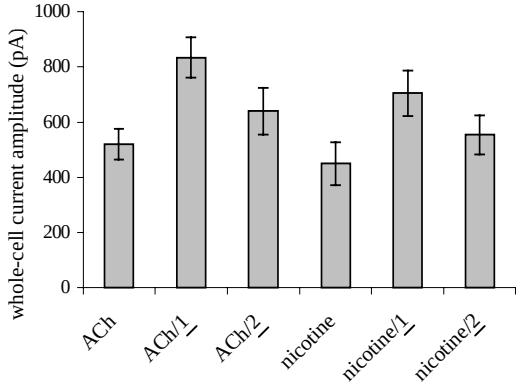

Fig.2: Whole-cell current measurements for the effect of 2 on human α4β2 nAChR subtype-expressing HEK-293 cells (the holding potential was set to –70 mV and 50 µM ACh or nicotine were co-applied with 1 and 2)

The current amplitudes induced by acetylcholine (ACh) or nicotine and further enhanced by 2 and 3 could be suppressed by the nicotinic antagonist mechamylamine. These findings suggest a nAChR-related mechanism. On the other hand, the calcium imaging method showed no increase of the α4β2 nAChR dependent Ca^{2+} influx for both fluoroalkylated derivatives (2, 3). However, under the same conditions 4 produced a 54% increase of the Ca^{2+} influx evoked by nicotine (Fig. 3).

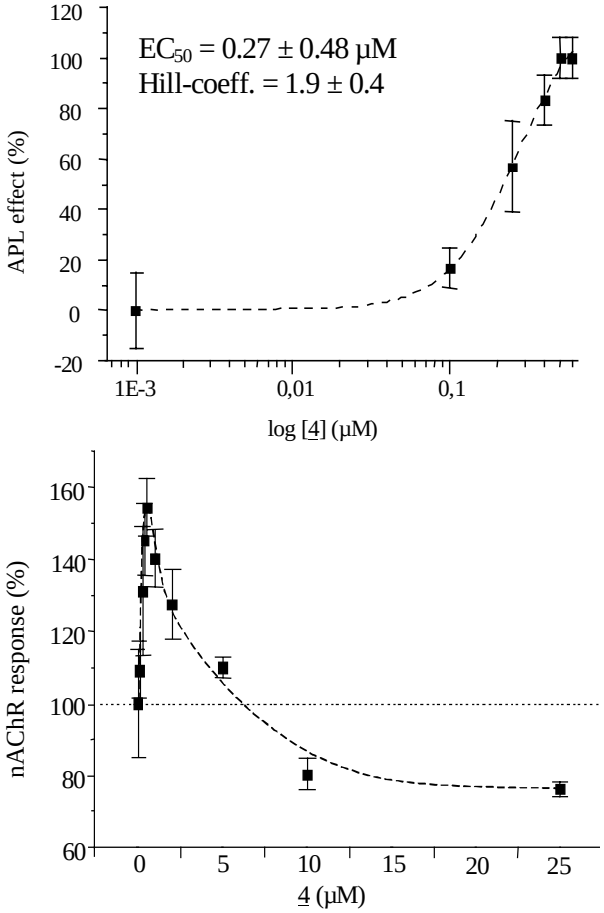

Fig.3: Dose-response curve for the effect of 4 on nicotine-activated Ca^{2+} influx into the human α4β2 nAChR subtype-expressing HEK-293 cells (after initial stimulation with 50 µM nicotine 0-25 µM 4 were co- applied); upper figure: APL-effect of 4 under same conditions

Conclusion: The galanthamine derivatives 2 and 3 are no likely candidates for intended PET studies and psychopharmacological examinations while 4 as a more potent allosterically potentiating ligand than galanthamine itself will be further developed. These results prompted us to tritiate 4 in order to conduct a binding assay which is currently being carried out.

[1] Schildan A. et al. Annual review 2000, previous article