MECHANISM OF THE EFFECT OF SINE-WAVE MAGNETIC FIELDS ON THE DESENSITIZATION OF THE 5-HT1B SEROTONIN RECEPTOR

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INTRODUCTION

A few studies performed on G-protein coupled receptors (GPCR) have shown that electromagnetic fields, in particular in the extremely-low-frequency (ELF) range (0-300 Hz), can induce changes in the properties of their binding to ligands. Massot et al., (2000) have shown that exposure to 50 Hz magnetic fields (MF) caused a reversible decrease of the 5-HT_{1B} serotoninergic receptor affinity in both rat brain membranes and cells transfected with the human 5-HT_{1B} receptor at field strengths above 0.6 mT. This effect was accompanied with a decrease in downstream signal transduction, demonstrated by the reduction of the agonist capacity to inhibit both forskolin-stimulated cAMP synthesis and K^+-evoked synaptosomal release of serotonin, also called 5-hydroxytryptamnine (5-HT).
In the present study, the 5-HT_{1B} receptor affinity was measured using saturation binding techniques. Using [^{3}H]5-HT, we first attempted to confirm that a 50 Hz MF exposure induces a decrease of the receptor affinity. Then, the role of both induced currents and static magnetic fields in the elicitation of the effect were assessed.

MATERIAL AND METHODS

Adult male Wistar rats (Charles River, Lyon, France) weighing 180-220 g at the time of their sacrifice were housed four per cage with food and water *ad libitum* and maintained in a temperature-controlled environment on a 12-hr/12-hr light/dark cycle. French regulations regarding animal care, animal handling, and experiments on live animals were followed.

R-(+)-8-hydroxy-2-(dipropylamino)tetralin (8-OH-DPAT), Tris, EDTA, phenylmethanesulfonyl fluoride (PMSF), aprotinin, pargyline, polyethylenimine (PEE), $CaCl_2$, serotonin (5-HT) and Guanosyl-Tri-Phosphate (GTP) were purchased from Sigma Aldrich (L'Isle d'Abeau-Chesne, France). (^{3}H)5-HT (80-130 Ci/mmol) was synthesized by Amersham Biosciences (Saclay, France).

The membrane preparation has already been described by Massot et al. (2000).

The main exposure system was composed of two identical sets of Merritt. The 96-well plates containing the rat brain membranes were placed in temperature-regulated containers located inside each set of coils, which could be independently activated. Samples were either exposed or sham-exposed in a randomised and blind fashion. This setup was also activated using two other generators to provide a static MF of 1.1 mT or a sine-wave MF at 400 Hz with an intensity of 0.675 mT_{rms}. Temperature and MF strength in each container were monitored in real time. Exposures lasted for 1 hour and were carried out at 25°C.

The protocol of Massot et al. (2000) was used with slight modifications. Rat brain membranes (300-500 µg ml^{-1}) were thawed and incubated with increasing concentrations of [^{3}H]5-HT in 96-well filtration plates (MAFB NOB, Millipore), pre-soaked with PEE 0.3%. Non-specific binding was determined using 10 µM of 5-HT. The binding to 5-$HT_{1B/1E/1F}$ receptors was determined in the presence of 0.1 µM 8-OH-DPAT to prevent binding to 5-HT_{1A} receptors. The binding to 5-$HT_{1E/1F}$ receptors was measured using 0.1 µM 8-OH-DPAT and 20 nM 5-CT to prevent binding to both 5-HT_{1A} and 5-HT_{1B} receptors, and 5-HT_{1B} binding represented the difference between these two bindings. Four wells were used for each condition.Incubation started just before the beginning of exposure and lasted 60 min. At the end of the incubation period, the multiwell plates were immediately filtered and the filters were washed twice with 100 µl of ice-cold incubation buffer. After drying, filters were placed in 4 ml of scintillation liquid and the radioactivity level was measured at least 3 hours afterwards using a LS-6000-SC Beckman counter. Five runs without exposure (i.e. sham-exposure vs. sham-exposure) were performed giving a coefficient of variation of 23% (SD/mean).

Current models of GPCRs activity have evolved from the ternary complex scheme into the extended ternary complex model (Kenakin, 2004), which we have used in this work (Fig. 1). This model refers to the receptor (R), activated by the ligand (A), and coupling to a G protein (G). In this scheme, two receptor states R_i (inactive state) and R_a (active state) coexist regulated by the equilibrium constant K_{act}. The G protein binds to the activated receptor state R_a to give a R_aG product with the equilibrium constant K_G. The ligand affinity is represented by K_A, the term α is a measure of the differential affinity of A for the activated receptor R_a, and the term γ a measure of the bound product AR_a for the G protein.

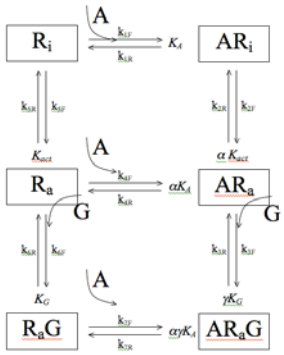

Figure 1: Scheme of the extended ternary complex model

Rigorous theoretical approaches (Kenakin, 2004) have related this model to the experimental capability to measure the apparent affinity, which is quantified by the equilibrium dissociation constant K_D. In the extended ternary complex model approach, K_D is related to the model parameters via the following equation:

$$K_D = \frac{1}{K_A}\frac{1+K_{act}(1+K_G[G])}{1+\alpha K_{act}(1+\gamma K_G[G])} \quad (1)$$

Results were obtained by first solving (1) and then the kinetic scheme.

RESULTS

Replication of the data of Massot

GTP modulation of the 5-HT$_{1B}$ receptor

In order to measure K_D of the low-affinity state of the 5-HT$_{1B}$ receptor, the substrate of the heterotrimeric G protein, GTP (10 mM), was used. Addition of GTP caused the expected decrease of the receptor affinity: K_D increased significantly from 1.41±0.09 to 6.94±0.80 nM (mean ± SEM).

However, Bmax did not vary (1673±138 CPM for the controls and 1703±196 CPM for membranes incubated with GTP). This shows that decoupling of 5-HT$_{1B}$ receptors from the G-protein does not cause an increase in the number of available binding sites.

5-HT$_{1B}$ binding under exposure to 50 Hz 1.1 mT MF

In saturation experiments using [^{3}H]5-HT, one-hour exposures at 1.1 mT_{rms} caused statistically significant increases in both K_D and Bmax (from 1.74±0.3 to 4.51±0.86 nM and from 1428±205 to 2137±399 CPM respectively; mean ± SEM; n = 12 ; excluding data points with low affinity: $K_{Dsham} > 4$ nM). These data suggest that there is a steep increase in K_D amplitude with MF strength in good agreement with the data of Massot et al. (2000).

5-HT$_{1E/F}$ binding under exposure to 50 Hz 1.1 mT MF

K_D was calculated for the 5-HT$_{1E/1F}$ receptor binding from the same set of runs and no effect of exposure was found: K_D was 15.0±2.1 and 15.5±2.8 nM for the sham-exposed and exposed samples, respectively (mean±SEM; n = 8).

Induced Currents

In a previous report, Massot et al. had shown that the effect on K_D was not due to induced currents. They had increased 12-fold the size of the dish containing the membranes and found no concomitant increase in the amplitude of the effect on K_D. We chose to confirm this finding by altering the frequency of the applied MF since the induced current density is proportional to both the diameter of the dish and the frequency. Experiments were thus performed at 400 and 0 Hz.

5-HT$_{1B}$ binding under exposure to 400 Hz 0.675 mT MF

The 5-HT$_{1B}$ specific binding was determined after one-hour exposures at 400 Hz 0.675 mT_{rms}. Under these exposure conditions, the density of current at the periphery of the 6-mm-diameter wells (0.98 mA m^{-2}) was almost 5 times larger than at 50 Hz 1.1 mT_{rms} (0.20 mA m^{-2}). However, there was only a small non-significant increase in K_D (1.57±0.22 nM for control samples and 2.65±0.82 nM for exposed sample; n = 6).

Comment: is there something missing ?

5-HT$_{1B}$ binding under exposure to static fields

Exposure to a static field (1.1 mT) did not induce major increases in K_D and Bmax. When data points corresponding to a low-affinity state ($K_{Dsham} > 4$) were discarded from the statistical analysis, there was a significant ($p = 0.048$) increase in K_D (2.00±0.19 nM and 2.91±0.32 nM for sham and exposed sample respectively; $n = 13$). However, there are data points below the "no effect line" indicating that the effect can be in the opposite direction, i.e. corresponding to an increase in affinity.

Modelling

In order to analyse specifically the 5-HT_{1B} receptor behaviour, the parameters of the model were first fixed, giving K_D values of 2 and 7 nM for [G] values of 5 and 0 nM, respectively (the latter corresponding to an excess of GTP). This set of initial conditions seemed suitable for further comparisons of MF exposure conditions. In order to reproduce the run-to-run variability of K_{Dsham} [G] was varied from 0.001 to 40 nM, as K_D is a function of [G] only making the hypothesis that the other factors in (1) do not vary from run to run.

Comment: A residual comment ??

A K_D sensitivity analysis to parameters variations was carried out in order to investigate which could be the parameters most affected by the field exposure. In particular, a strong increase in K_D to 9 nM corresponded to a decrease of γ from 4 to 0, whereas, in the presence of GTP ([G] = 0), K_D depended mainly on α (with $\alpha = 3$ in unperturbed control conditions). There is a good agreement between the fit of the experimental data and the modelling at 50 Hz with $\gamma = 0.9$ and $\alpha = 3$.

DISCUSSION AND CONCLUSION

Replication of the Massot et al. Results

The replication of the data of Massot et al. (2000) has been successful, as similar increases in K_D and Bmax at around 1 mT were observed independently in the two laboratories, using the same exposure system. Massot et al (2000) found a steep rise in K_D as a function of MF strength and a plateau above 1 mT. This is well modeled using our kinetic scheme: under the hypothesis of a linear decrease of γ with field strength, Fig. 2 shows an increase in K_D that perfectly matches the observation of Massot et al. in their Fig. 2 (2000). At field strength above 1 mT the cancellation of γ limits a further increase of K_D and results in a plateau.
In the same experiments performed at 50 Hz 1.1 mT, the binding characteristics of the 5-$HT_{1E/1F}$ receptors were not affected by exposure, in agreement with the findings of Massot et al. (2000). Therefore, the conclusion of these authors that only the 5-HT_{1B} receptor is responding to the field is reinforced.
Overall, this set of results on the binding of the 5-HT receptor under exposure to 50 Hz 1 mT field constitutes one of the few replicated observations of an effect of ELF MF in the mT range.

Induced Currents

One of the main questions regarding bioeffects of ELF-MF is whether the magnetic field or the induced currents are acting on the biological systems. Evidence is given in our 400 Hz experiments that induced current are not the cause of the effect of exposure on binding. This is in agreement with the report of Massot et al. showing no increase of the amplitude of the effect, at low field strength, when using large Petri dishes. The difference in the amplitude and nature of the effects observed using AC (50 and 400 Hz) and DC fields is thus not due to different current densities but to other field characteristics.

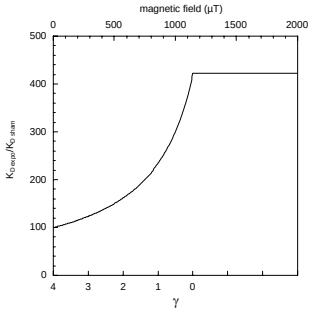

Figure 2: Modelling of the dependence on MF strength of the increase in K_D based of the Massot et al. (2000) data.

It is hypothesized here that the polarity of the signal and not the induced current is the key factor: the "monopolar" DC

field has an effect on γ and α while the AC bipolar 50 and 400 Hz fields have an effect on γ but not on α as if the effect of the two polarities on that parameter cancelled each other.

Comment: 2&3 or 4 &6 ? i cannot understand why 3&4

Mechanism

In the light of our findings, many of the reported effects of static, ELF and pulsed MF on biological effects that involve GPCRs may be "revisited". For example, Varani et al. (2002, 2003) found no effect on affinity after ELF-EMF exposure. However, these studies were carried out using antagonists, which, in contrast to agonists, bind to the receptors regardless of their state of coupling to G-protein.
As receptor activity states are dependent on the environment surrounding the receptor, membrane fluidity can modulate both receptor affinity and concentration (Pucadyil and Chattopadhyay, 2004). The influence of the fluidity on the MF effect on binding will thus be studied by varying the cholesterol content of the membrane. Further work on the effects of MF on 5-HT_{1B} receptor binding will include the use of another specific agonist [^{3}H]5-CT, and the study of the influence of the presence of GTP, that is for [G] = 0.
In conclusion, the successful replication of previous findings showing an effect of AC MF on the 5-HT_{1B} receptor warrants further studies on other GPCRs, in view of their ubiquitous nature and major role in biology. The modelling part of this work will contribute to a better understanding of the effects of ELF and static fields in the mT range.

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