**Waking Up Anna**

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**Part 2: A Clue in Anna’s Spinal Fluid**

Although we have all may have been sleep deprived at some point in time and can relate to how difficult it becomes to focus or carry out simple routine tasks, Anna Sumner’s case was different – she craved sleep and would not be able to wake up refreshed even after ~30 hours of sleep.

Before discussing Anna’s case any further let us review “Neurochemistry of Sleep” that you learned about in part C. of the Preparation section of this case.

Review the structure and function of GABA-A receptors and some key vocabulary terms used in the case (Agonist, Antagonist, Allosteric modulators). The relevant sections to review are included below for your convenience.

* Structure and Function of GABA-A receptors:
	+ Structure: These receptors are pentameric, i.e., they are composed of 5 transmembrane protein subunits that together form a chloride ion-selective channel. Although there are many different isoforms of the protein, the most common type of GABA-A receptors found in the human brain are composed of 2 alpha (**α**), 2 beta (**β**), and 1 gamma (**γ**) subunit.
	+ Function: Binding of neurotransmitters triggers ion channels to open. When GABA binds to the GABA-A receptor, the ion channel is open and chloride ions can cross the cell membrane. Other ligands, including clinically important drugs, *e.g.* benzodiazepines (a sedative), anesthetics and depressants can also bind to the GABA-A receptor to alter its function by opening or closing the Cl− channel. A simple cartoon of the GABA-A receptor is shown in Figure 2 along with the binding sites of various ligands.



Figure 2: GABA-A receptor, chloride (Cl−) ionophore complex. The cut-away view shows binding sites for a variety of compounds that influence the function of the receptor complex. (Adapted from Olsen, R.W. and DeLorey, T.M., (1996), GABA Receptor Physiology and Pharmacology, Basic Neurochemistry: Molecular, Cellular and Medical Aspects. 6th edition.)

* Key vocabulary often used in describing the binding of drugs and small molecules to their target receptors is included in Box 2.

*Box 2: Vocab*

**Agonists**: These are drugs or molecules that turn on the function of a protein. The natural ligand usually acts as an agonist.

**Antagonists**: These are drugs or molecules that decrease or inhibit the function of a protein.

**Allosteric modulators**: The word root “allo” means “other”. These are drugs or molecules that bind at a location other than the natural ligand’s binding site. Modulators could be positive (increase activity of the protein) or negative (decrease its activity).

Back to Anna’s case - After excluding known causes of sleepiness, doctors stopped all stimulants and drugs that were being prescribed to Anna to collect a sample of her cerebrospinal fluid (CSF). The doctors were looking for clues about the unique pharmacology within her sleepy brain. Researcher scientists analyzed her sample along with 31 similarly hypersomnolent patients and found a substance that increased GABA-A receptor function in cultured cells!

Let’s examine some key evidence that scientists gathered to help understand Anna’s case.

*Box 8: Experimental techniques*

Ions, like Na+, K+, Ca2+, and Cl−, are present in different concentrations inside and outside neuronal cells leading to a **membrane potential** difference in charge. When channels are open, ions can move across cellular membranes according to the concentration and electrical gradients, leading to rapid and transient changes in distribution of these charged particles. Movement of ions is recorded as **current** and these electrical signals are transmitted through neurons to trigger physiological changes.

**Patch clamp** is an experimental technique used to measure changes in electrical properties of cell membranes to determine whether or not the channels in these receptors are open or closed in the presence of different ligands.

When GABA-A receptor channels are closed there is a baseline reading. In these experiments, GABA and/or other GABA-A receptor agonists open the chloride channels to let negative ions (Cl−) into the cells. The patch clamp records this entry of Cl− ions as a downward deflection from the baseline. The larger the deflection (or dip) in the recording the more the Cl− ions have entered, inhibiting the neuron.

Scientists used patch clamp electrical recordings on a specific cell line with GABA-A receptors to measure currents triggered by the following:

|  |  |  |
| --- | --- | --- |
| **Trace**  | **What was added** | **Why was it added** |
| a | 10 μM GABA alone | control to establish a baseline |
| b | Hypersomnolent patient CSF alone | To see if the CSF factor can work alone |
| c | Hypersomnolent patient CSF + 10 μM GABA | To see if the CSF factor impacts GABA binding and GABA-A receptor function |
| d | Flumazenil, a competitive antagonist + Hypersomnolent patient CSF + 10 μM GABA  | To see if the CSF factor binds to the benzodiazepine binding site |
| e | Flumazenil, a competitive antagonist + 10 μM GABA | Control to see that Flumazenil does not interfere with GABA binding |
| f | normal GABA concentrations in typical CSF | Control after all pharmacological agents were washed away |

Review the chloride currents seen for each of these cases and answer the following questions.



Figure 3. Chloride currents recorded from HEK293 cells expressing human GABA-A receptor. Vertical axis (current) calibration bars equal 200 pA (picoAmps) and horizontal axis (time) calibration bars equal 5 s.

Q1 (2 pts). Compare the chloride current traces shown in b. and c. and explain the conclusion that can be drawn from your observation.

Ans: Trace b investigates the hypothesis that Anna’s CSF factor could be excess GABA, resulting in hypersomnolence. Since the presence of the CSF factor alone did not show any current flow, we can rule out the hypothesis that there is additional agonist in patient CSF.

Trace c shows increased function with patient CSF in the presence of GABA, suggesting activity similar to a positive allosteric modulator.

Q2 (2 pts). Compare the chloride current traces shown in c. and d. and explain the conclusion that can be drawn from your observation. *Hint: Also compare with trace a.*

Ans: Flumazenil is a known competitive antagonist at the benzodiazepine site. Trace d shows that flumazenil reverses the effect of patient CSF. This provides evidence that something in the patient CSF is acting like a positive allosteric modulator, more specifically, like a benzodiazepine.

Q3 (1 pt). Based on the traces in Figure 3, which of the following statements is correct? (Circle or highlight one.)

1. Only in trace c. the GABA-A receptor is open and Cl− ions are flowing into the cell
2. The substance in Anna’s CSF can bind to GABA-A receptor and open the Cl− channel
3. The substance in Anna’s CSF needs GABA in order to open the Cl−channel
4. Flumazenil can close the GABA-A receptor Cl− channels
5. When the GABA-A receptor is open K+ ions are flowing out of the cell

Q4 (1 pt). Based on the results presented, speculate as to where in the GABA-A receptor do you think the CSF factor binds relative to other molecules binding to the receptor? Briefly explain your answer.

Ans: It probably binds to the same site where the positive allosteric activator (benzodiazepine).