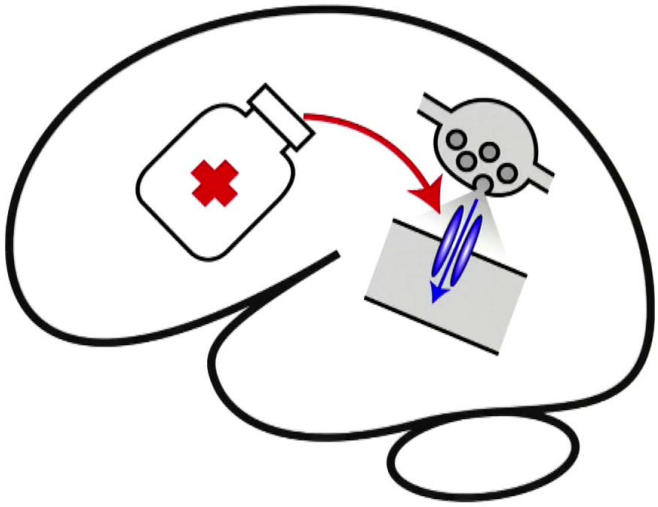
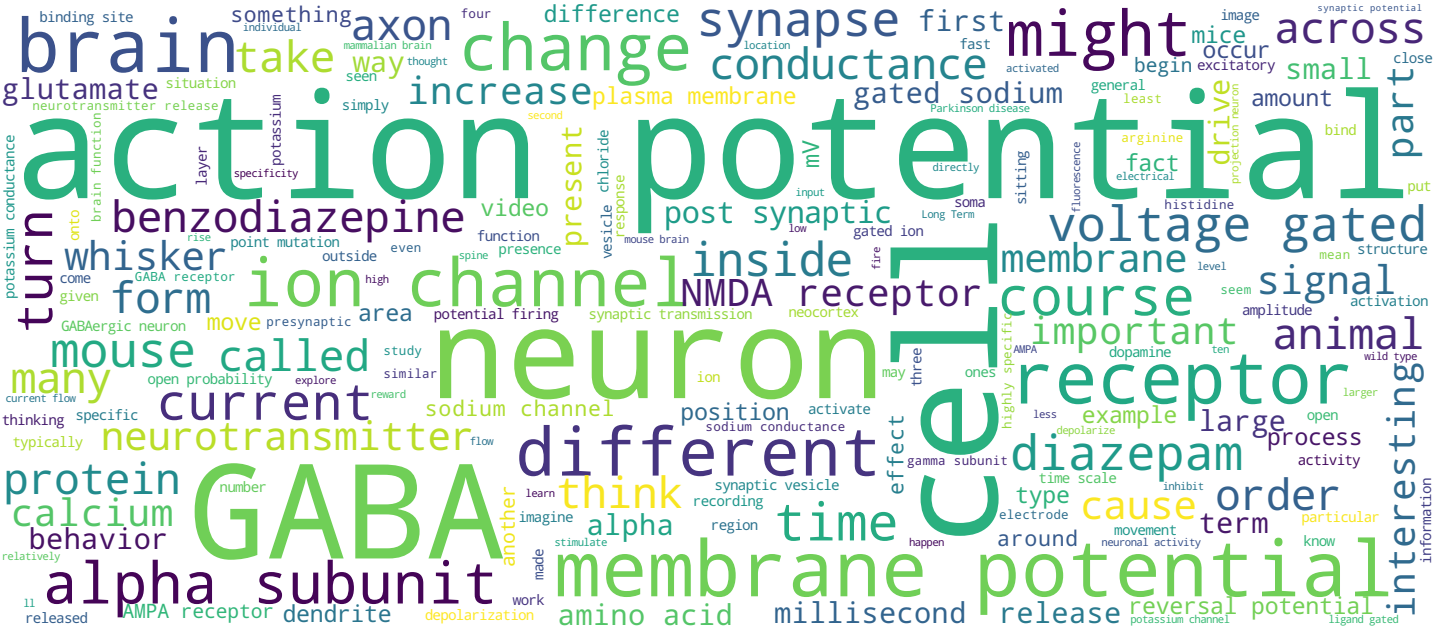


5.3 Benzodiazepines

Cellular Mechanisms of Brain Function

Prof. Carl Petersen



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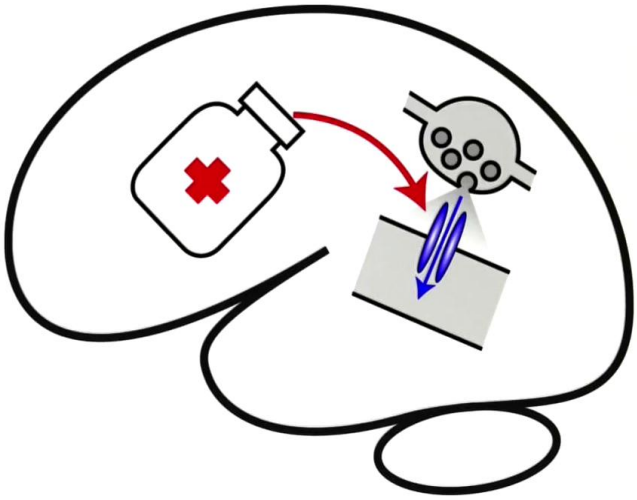
Video

EPFL

Benzodiazepines act upon $GABA_A$ receptors



Cellular Mechanisms of Brain Function

Neuropharmacology is a very important field of neuroscience. It forms the basis of the pharmaceutical industry and our hopes for treating brain diseases. There are many compounds that act specifically at different receptors in the brain. So for AMPA receptors we have CNQX and MBQX for NMDA receptors we have APV and MK-801, and for the GABA-A receptor we have antagonists like picrotoxin, bicuculline and gabazine. These antagonists have highly specific actions and are extremely useful in the experimental setting, but therapeutically, they've not proven to be useful. In this video we're going to look at benzodiazepines that have turned out to have interesting therapeutic potential and have been extensively used. Benzodiazepines were first developed in the '60s, and were marketed as Valium, also known as diazepam, and since then many benzodiazepines have been developed with higher specificities and better therepeutic outcome. It's interesting to look at the benzodiazepines, because they have an extremely specific mechanism of action and a lot is known about how they function at the molecular level. That's what we'll explore in this video.

Notes

Summary

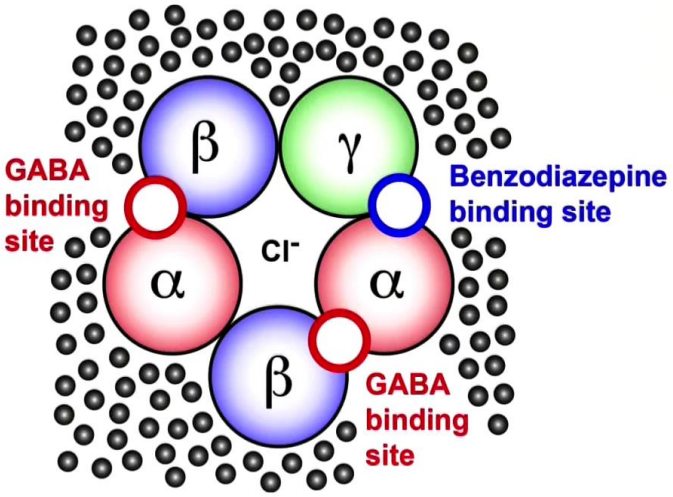
0m 05s

Structure of $GABA_A$ receptors

$GABA_A$ receptor genes

α1, α2, …, α6
β1, β2, β3, β4
γ1, γ2, γ3
ρ1, ρ2, ρ3, δ, π, ε, θ

$GABA_A$ receptor



Cellular Mechanisms of Brain Function

Let's begin by taking a closer look at the GABA(A) receptor. The GABA(A) receptor is a pentameric structure, made out of five different subunits, each of which is an individual protein that comes together to form the ligand-gated GABA(A) receptor with the chloride channel down the middle. Each subunit is encoded by a single protein and there are a number of different genes that code for those subunits. They can be divided into different families. There's an alpha, beta, gamma, rho, delta, pi, epsilon, theta — types of GABA(A) receptor genes, and in general for most GABA(A) receptors, they're composed of two alpha subunits, two beta subunits and a gamma subunit. The gamma subunit can also be one of these rho or other ones in grey. The GABA binding site turns out to be somewhere between the alpha and the beta subunit. So in fact, there are two GABA binding sites between each of the alpha and beta subunits. The benzodiazepine binding site, however, is at a different location and sits between the alpha and the gamma subunits. In fact, you need to have a gamma-2 or a gamma-3 subunit present in the GABA receptor in order for the benzodiazepines to bind.

Notes

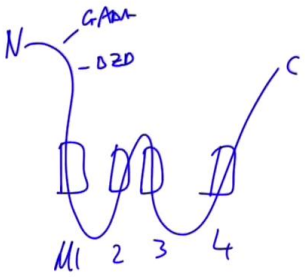
Summary

1m 24s

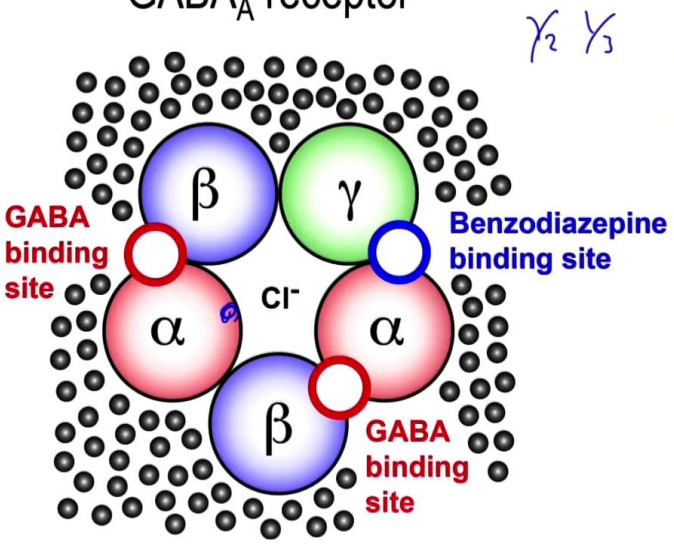
Structure of $GABA_A$ receptors

$GABA_A$ receptor genes

α1, α2, …, α6
β1, β2, β3, β4
γ1, γ2, γ3
ρ1, ρ2, ρ3, δ, π, ε, θ



$GABA_A$ receptor



Cellular Mechanisms of Brain Function

In terms of the amino acid structure of the GABA(A) receptor, they form a large N-terminal region where the GABA binding site is encoded and also there's a benzodiazepine binding site. We have four transmembrane alpha helices — one, two, three and four — and this one here turns out to be the [paw]-forming region which then hosts the chloride ion channel. The GABA and the benzodiazepine binding sites are sitting here on the N-terminal which is on the outside of the cell.

Notes

Summary

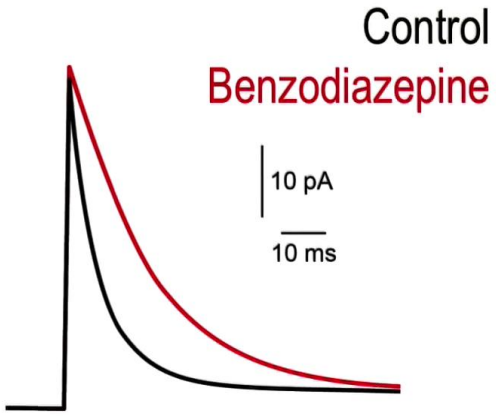
2m 50s

Benzodiazepines potentiate $GABA_A$ currents

Benzodiazepines do not activate $GABA_A$ receptors on their own, but only potentiate GABA-evoked currents.

Benzodiazepines increase the affinity of GABA for binding to the $GABA_A$ receptor.

Benzodiazepines prolong the duration of IPSCs.



Cellular Mechanisms of Brain Function

The benzodiazepenes don't in themselves activate the GABA-A receptors, so they bind to the GABA-A receptor but it's at a different site where GABA activates the ion channel. What the benzodiazepines do is that they increase the affinity of the GABA-A receptor for GABA. So GABA binds more tightly to it in the presence of benzodiazepines. If we think about the electrophysiology, functionally relevant in terms of the IPSCs mediated by synaptic transmissions — that's inhibitory postsynaptic currents — then we'll see that under control conditions the IPSC has a certain amplitude and a certain duration. If you then apply benzodiazepine, that then doesn't change the amplitude of the IPSC, but it prolongs the duration of the IPSC. So there's a total charge transfer that's mediated in the presence of benzodiazepines that's larger, and so it potentiates the GABA-A currents and potentiates the inhibition in the brain. So benzodiazepines have an interesting action. They don't in themselves cause inhibition, but they only potentiate the effect of GABA when it's normally released in the brain. So they only act when they GABA's been released at the right time and the right place in the brain. So it's a highly specific potentiation of GABAergic inhibition that the benzodiazepines mediate.

Notes

Summary

3m 32s

Amino acid sequences of $GABA_A$ receptors

Not all $GABA_A$-α subunits are sensitive to benzodiazepines. Benzodiazepines potentiate $GABA_A$ receptors containing the α1, α2, α3 or α5 subunits, but it has no effect upon α4 /α6.

Amino acid sequences:

α1	94	WTPDTFFHNGKKS	106
α2	94	WTPDTFFHNGKKS	106
α3	119	WTPDTFFHNGKKS	131
α4	92	WTPDTFFRNGKKS	104
α5	98	WTPDTFFHNGKKS	110
α6	93	WTPDTFFRNGKKS	105

Cellular Mechanisms of Brain Function

There's further specificity as to which type of GABA-A receptors the benzodiazepines act upon. It turns out that GABA-A receptors need to contain alpha-1, -2, -3, or alpha-5 subunits and if instead they have an alpha-4 or alpha-6 subunit, then the benzodiazepines don't have any effect. Now as the molecular biologist were beginning to clone and sequence the different GABA receptor alpha subunits, they found that there was in general very high homology, so that the amino acid sequences of the different alpha subunits were very similar, but there were of course also many differences. Here in one particular sequence, alignment of the different subunits, we see that the four that bind benzodiazpines have a histidine at this position — 101 for the alpha-1 and alpha-2 subunits, so these are the numbers counting from the N-terminal, the amino acids, so we have the different amino acids here, and at position 101 in both alpha-1 and alpha-2 we have a histidine. Alpha-4 and alpha-6 in this equivalent position have an arginine that's present. So there's a difference in the amino acid structure of the receptors that bind benzodiazepines and the ones that don't.

Notes

Summary

5m 00s

Amino acid sequences of $GABA_A$ receptors

Not all $GABA_A$-α subunits are sensitive to benzodiazepines. Benzodiazepines potentiate $GABA_A$ receptors containing the α1, α2, α3 or α5 subunits, but it has no effect upon α4 /α6.

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α6	93	WTPDTFFRNGKKS	105

Cellular Mechanisms of Brain Function

There are of course other differences in the amino acid sequences of the GABA-A receptors, but you can at least test to see whether this makes the difference for benzodiazepine binding. So we can take the alpha-1 or the alpha-2 subunits and change this histidine to an arginine and now see: Does that make a difference to the benzodiazepine effect?

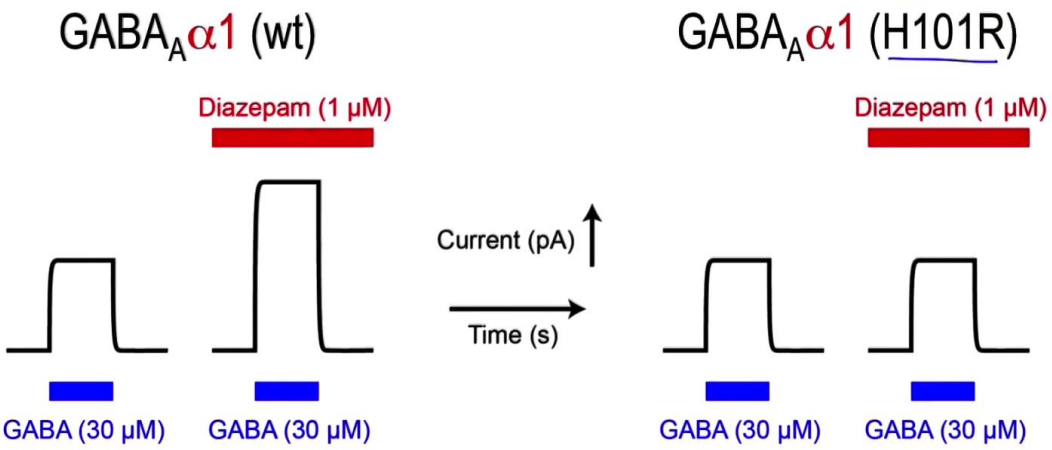
Notes

Summary

6m 27s

Point mutations in $GABA_A$ receptors

Molecular biologists have discovered how to make genetic mutations.
In the laboratory, we can therefore change **H** (histidine) to **R** (arginine).



Cellular Mechanisms of Brain Function

The molecular biologists have figured out how to make changes to the amino acid sequence of GABA-A receptors and they've also figured out how to express them in heterologous systems and so here we're expressing wild type GABA, alpha-1 subunit together with a beta and a gamma subunit, express them, apply GABA, and we see a nice GABA-mediated chloride current. In the presence of diazepam — that's the benzodiazepine Valium — we apply that at one micromolar and now when GABA is applied the GABA-mediated currents are much larger. That's the potentiating effect of benzodiazpines upon the GABA-A receptor. Now, in the mutants where we take the histidine at position 101 and change it to an arginine — that's what's typically present in the alpha-4 and alpha-6 subunits — these are the ones that are insensitive to diazepam — now we change just this one amino acid in the alpha-1 subunit and now when you apply diazepam there's no potentiation of the GABAergic currents. It's the same in the presence of diazepam as it is without diazepam when this point mutation has been carried out. So it turns out that this histidine in position 101 is essential for the diazepam, in order to mediate its effect at potentiating the GABA-A receptor.

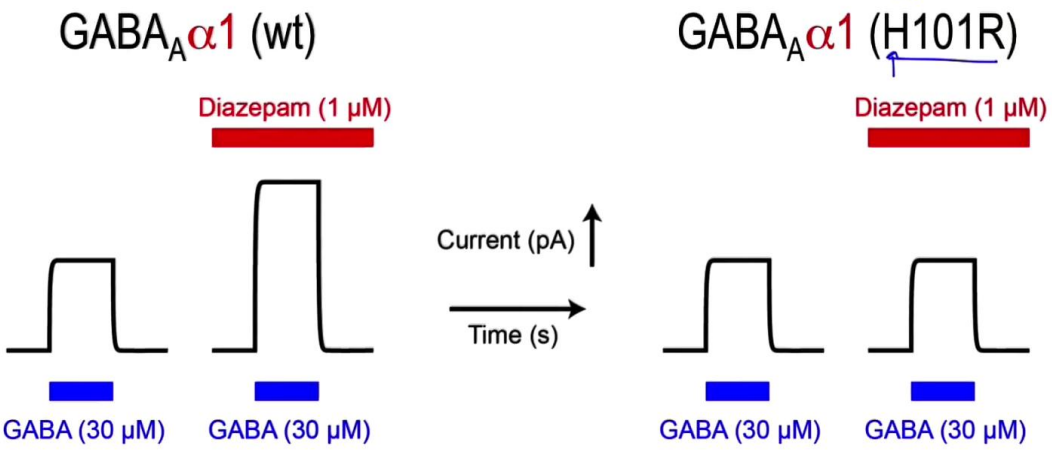
Notes

Summary

6m 48s

Point mutations in $GABA_A$ receptors

Molecular biologists have discovered how to make genetic mutations.
In the laboratory, we can therefore change **H** (histidine) to **R** (arginine).



Cellular Mechanisms of Brain Function

Interestingly, this point mutation makes no difference to the currents evoked by GABA in the absence of diazepam. So in many respects, this point-mutated GABA alpha-1 receptor is identical to the wild type, except in the presence of the exogenous agent diazepam or other benzodiazepines. This then allows us to do very interesting genetic testing to see which subunits mediate which actions of the benzodiazepines.

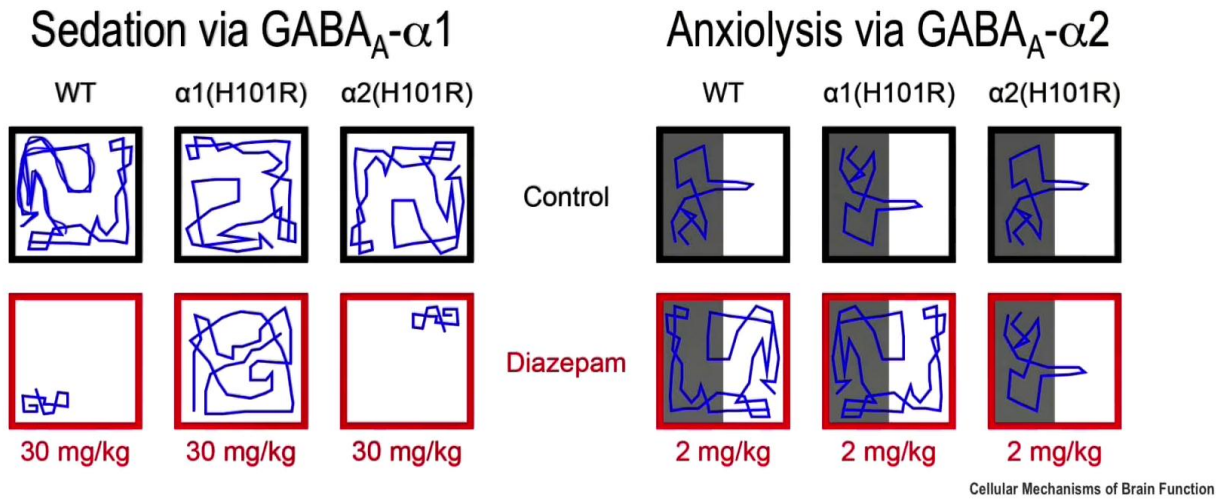
Notes

Summary

8m 16s

Mutating $GABA_A$ receptors in the mouse genome

Through genome editing, we can change **H** (histidine) to **R** (arginine) in specific subunits of $GABA_A$ receptors in living mice.



So in the mouse genome, we can also go and make exactly the same mutations to the GABA-A receptors. We can change — in the alpha-1 or the alpha-2 subunits — we can take that histidine at position 101 and change it to an arginine and then we'll make GABA receptors for these specific subunits that are insensitive to diazepam. What's good about doing this at the genome level in mice is that we can then see: What are the behavioral effects of applying the drug — the benzodiazepine — to an animal, in terms of the behavioral change that diazepam makes. We're interested in two different effects of the benzodiazepines. One is the sedative action, and that's what we'll look at first. If you take a wild type mouse and you put it inside an arena, you can then track the position of that mouse as it runs around. You can see where it goes and you can quantify the distance that it moves. If you then give that same mouse some diazepam at a relatively high concentration — 30 mg/kg — that has a sedative action on the mouse. The mouse might go to sleep, or it will just relax and do nothing, perhaps staying in this corner of the cage.

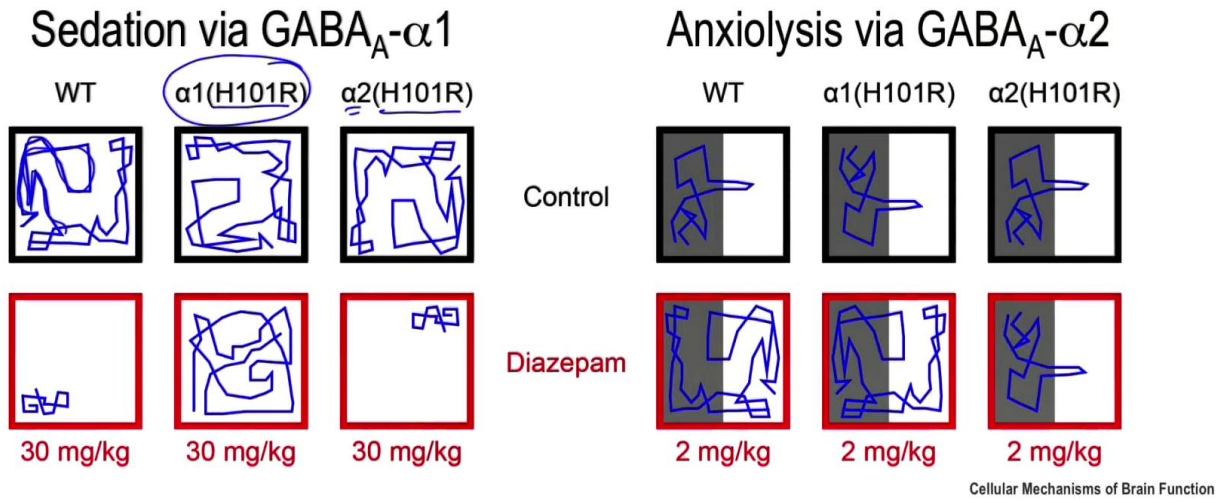
Notes

Summary

8m 48s

Mutating $GABA_A$ receptors in the mouse genome

Through genome editing, we can change **H** (histidine) to **R** (arginine) in specific subunits of $GABA_A$ receptors in living mice.



Cellular Mechanisms of Brain Function

If you now take a different mouse where you've made the genetic mutation so that the alpha-1 subunit is no longer sensitive to diazepam, then the basal condition, where there's no benzodiazepines present, the animal is just like wild type, the GABA alpha-1 subunit behaves just like the normal alpha-1 subunit in the absence of diazepam, and so the mouse runs around, explores its environment. You give it the large dose of diazepam and in the point-mutant mouse for the alpha-1 subunit, it has no effect. The sedative effect of diazepam is gone. So apparently, sedation is mediated by the alpha-1 subunit of the GABA-A receptor. You can test the specificity for it acting on the alpha-1 subunit by doing the same mutation in the alpha-2 subunit. So now we take a point-mutant mouse, it has the histidine replaced by an arginine in position 101 for the alpha-2 GABA-A receptor, we give the diazepam, and the sedative action still works. So sedation doesn't work via the alpha-2 subunit, but it's specifically mediated by the alpha-1 subunit of the GABA-A receptor — a remarkable specificity. The other interesting action of the benzodiazepines is anxiolysis, a reduction in anxiety.

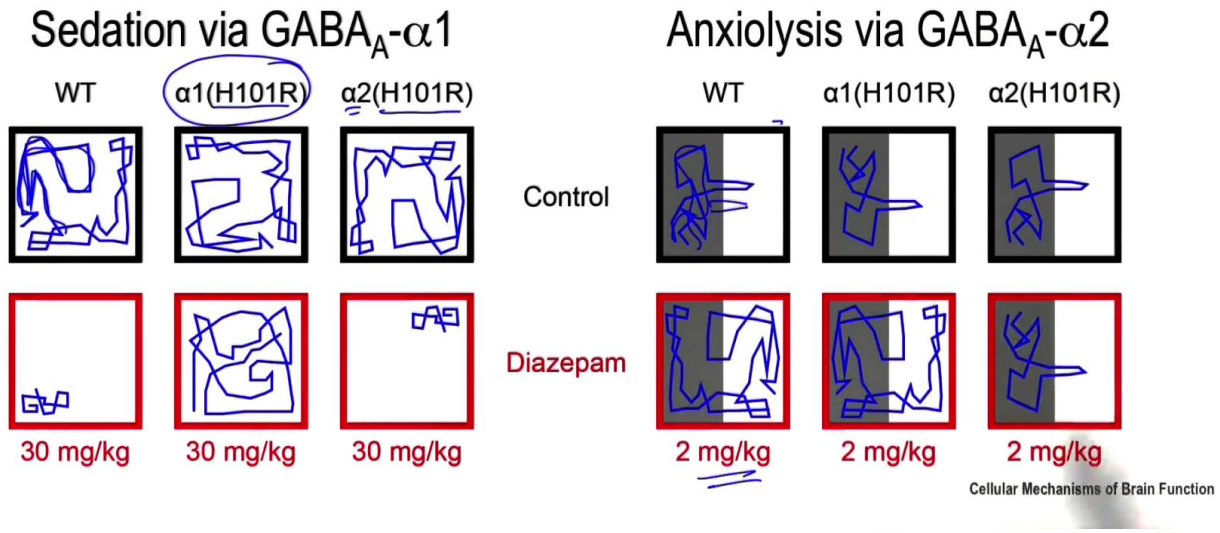
Notes

Summary

10m 06s

Mutating $GABA_A$ receptors in the mouse genome

Through genome editing, we can change **H** (histidine) to **R** (arginine) in specific subunits of $GABA_A$ receptors in living mice.



This we can also monitor in mice by thinking about their overall behavior and what they're afraid of. Mice are worried about large, open, light spaces. They're nocturnal animals and they prefer to move around in the dark. So experimentally in the lab, we can design behavioral arenas that have a dark area and a light area. If you put the mouse in this arena, it will tend to run around in the dark area. Every now and then, it might poke into the light area, but it's afraid, it's anxious, and it will rapidly return to the dark area. If you give these mice a small dose of diazepam, then they'll relax, and their fears will be reduced; they're less anxious. They'll then go and explore the light area to a much greater extent than under the control conditions. That's the anxiolytic effect of diazepam, here occurring at much lower doses than the high concentrations for sedation. We can then do the same two point mutations as before and study the effects of diazepam. In the alpha-1 point mutation, the diazepam still works. It has the anxiolytic effect, and the mouse will explore the light area. But in the alpha-2 point mutation the diazepam fails to have the anxiolytic effect.

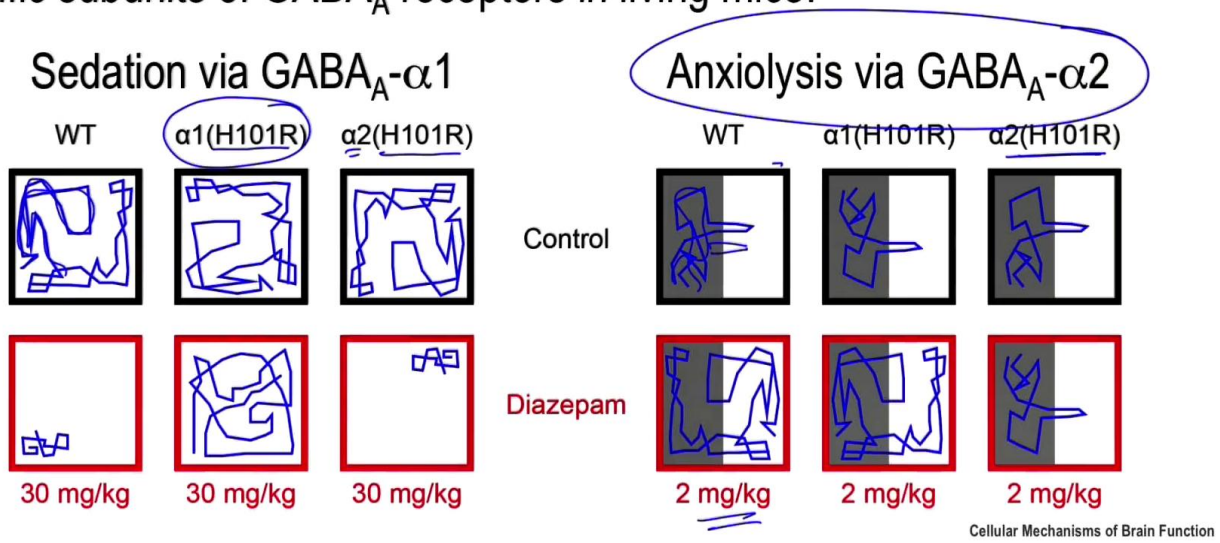
Notes

Summary

11m 35s

Mutating $GABA_A$ receptors in the mouse genome

Through genome editing, we can change **H** (histidine) to **R** (arginine) in specific subunits of $GABA_A$ receptors in living mice.



The point mutation here means that the diazepam can't act upon the alpha-2 subunit and the fact that there's then no anxiolytic relief from diazepam means that anxiolysis is being mediated by the alpha-2 receptor, at least in mice.

Notes

Summary

12m 57s

Benzodiazepines

- Benzodiazepines act upon specific subtypes of $GABA_A$ receptors.
- Benzodiazepines acting upon $GABA_A$-α1 receptors mediate a sedative effect in mice (sleep).
- Benzodiazepines acting upon $GABA_A$-α2 receptors mediate an anxiolytic effect (anti-anxiety).

Cellular Mechanisms of Brain Function

So we've seen that benzodiazepines act in a highly specific way upon some some subtypes of GABA-A receptors. It turns out that the sedative action of benzodiazepines is mediated by the alpha-1 receptor and the anxiolytic effect of benzodiazepines is activated via the alpha-2 receptor. That, of course, is an analysis that's been done in mice, and it's unclear to what extent that will carry over into the human situation, but it has of course inspired the pharmaceutical industry to go and make agents that are specifically acting at alpha-1 and alpha-2 subunits, and of course, it's probably also interesting to explore the other subunits of the GABA receptors that may have different therapeutic actions.

Notes

Summary

13m 17s