Research
Based
Curricula

Your brain on estrogen

Key Stage 5

Biology/Psychology

2020

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Resource One Model Answers

Answers

1. Aromatase.
2. Cholesterol is transported to the inner mitochondrial membrane by steroidogenic acute regulatory protein (StAR). This is the rate-limiting step of steroid synthesis. Next, cholesterol is converted to pregnenolone by the cytochrome P450 side chain cleave (P450scc) enzyme, which cleaves cholesterol's side chain.
3. Without the 3β-HSD enzyme, the hormones you would be able to make are:
    - Pregnenolone
    - 17-hydroxypregnenolone
    - DHEA
4. To compare the effects of androgens and estrogens, you could use DHT as the androgen instead of testosterone. This is because DHT cannot be aromatised to estrogen, so you can be sure that any effect you see is completely due to androgen. The experiment might involve treating one animal with DHT (androgen) and one animal with estradiol (estrogen).

Resource One Model Answers

Answers

5. The hypothalamus, pituitary and gonads are all related in the hypothalamic-pituitary-gonadal (HPG) axis. The axis starts with the hypothalamus signalling to the pituitary by the release of gonadotropin-releasing hormone (GnRH). GnRH acts on the anterior lobe of the pituitary glad, stimulating it to produce and release luteinising hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH travel in the bloodstream to reach the gonads, whether that be the testes or ovaries. At the testes, LH and FSH stimulate the release of the testosterone, which feeds back to the hypothalamus and pituitary to tell it to stop releasing GnRH and LH and FSH. This negative feedback loop is also seen in the ovaries, where LH and FSH stimulate the production of estrogen which feeds back to the brain in a similar way to testosterone.
You may also draw a diagram illustrating negative feedback loops to aid this answer.

Resource Two Model Answers

Answers

1. ERα and ERβ are part of the nuclear receptor family, and as such are expressed inside cells, within the cytoplasm near the nucleus. Estrogen is able to access ERα and ERβ because it is a steroid hormone. All steroid hormones are lipophilic, allowing them to easily diffuse through the plasma membrane to reach nuclear receptors.
2. An estrogen response element (ERE) is a small sequence of DNA found in the promoter region of a gene. The promotor region is the sequence of DNA that initiates the transcription of a gene. The estrogen response element is where the estrogen receptor dimer binds to initiate transcription of target genes.
3. *See table on next page.*

Resource Two Model Answers

Answers

3. I would expect an essay-style answer but the table shows the talking points you could include:

Variable	Genomic signalling	Nongenomic signalling
Receptors	Nuclear: ERα and ERβ	Membrane: Isoforms of ERα and ERβ (such as ERα-36) and GPER1
How does estrogen access the receptor?	By diffusing through the cell and binding nuclear receptors in the cytoplasm	By binding the receptor at the cell membrane
Does the receptor form a dimer?	Yes – nuclear receptors can form homodimers (e.g. ERα + ERα or ERβ + ERβ) or heterodimers (ERα + ERβ)	No (or not as far as we know from current research)
Any second messengers?	No	Yes – a cascade of molecules, proteins and cell signals. Some of these second messengers are kinases (e.g. PKA) and can phosphorylate other molecules to either activate or deactivate them (e.g. CREB1).
How is the response brought about?	Estrogen receptor dimer acts as a transcription factor by translocating to the nucleus and binding and ERE to initiate gene transcription	Second messengers bring about the cell's response
Direct or indirect mechanism?	Direct	Indirect
Timeframe	Longer – minutes to hours	Shorter – seconds to minutes

Resource Two Model Answers

Answers

4. The primary antibody binds antigens present on the protein of interest. The secondary antibody is conjugated to a fluorescent protein called a fluorophore. The secondary antibody binds the primary antibody, and the colour of the fluorophore can be seen under a microscope. This allows us to see where proteins are distributed in a tissue.

5. For the needs of this question, you can get away with using a single modulator! You could use any of:

    - tamoxifen (antagonist of ERα and ERβ; agonist of ERα-36 and GPER1)
    - ICI-182,780, also called Faslodex™ (antagonist of ERα and ERβ; agonist of ERα-36 and GPER1)
    - E_2-BSA (membrane-limited form of estrogen, so is not able to pass through cells to bind ERα or ERβ and can only bind membrane receptors such as ERα-36 or GPER1)

Resource Three Model Answers

Answers

1. Resting membrane potential = -70mV
   Threshold potential = -55mV
   Peak of the action potential = +30mV
   Hyperpolarisation = any value below (more negative than) -70mV

2. Statements should go in the following order, with missing words:
   d. into/inside; depolarisation
   a. threshold potential; all-or-nothing
   c. into/inside; axon
   f. close; into/inside; open; out
   e. hyperpolarisation
   b.

3. Nuclear receptors ERα and/or ERβ. This is because the effect of estrogen is completely blocked by ICI-182,780, which inhibits nuclear receptors ERα and ERβ but would normally stimulate membrane receptors like ERα-36 and GPER1.

4. Typical neurotransmitters are synthesised in advance and stored in neuronal vesicles. Estrogen is lipophilic, so cannot be stored within vesicles because it would just diffuse out of them! The release of typical neurotransmitters depends on vesicles fusing with the membrane. The release of synaptocrine estrogen depends on the activity of aromatase.

5. Dendritic spines are rapidly modulated by sensory experiences, learning and development. They can be stimulated to grow (a process called spinogenesis), or if the stimulus is taken away, the spines are re-internalised. Therefore, you must have continuous exposure to a stimulus in order for dendritic spines to remain present.

Resource Four
Model Answers

Answers

1. Any answer that correctly demonstrates the ideas that:
    - During perinatal development, the brain is organised either by a lack of estrogens (as in the female brain) or estrogens that have been aromatised from testosterone (as in the male brain).
    - The organisational effects of perinatal development are permanent, affecting behaviours even in adulthood.
    - The neural structures that were organised during perinatal development are then activated during adulthood, being acted upon by gonadal hormone secretions as a result of sexual maturation and the initiation of signalling via the hypothalamic-pituitary-gonadal (HPG) axis.
2. The ovaries are quiescent during perinatal development. The ovaries provide the body (and the brain) with the majority of estrogen. Also, females treated prenatally with testosterone showed less female behaviours and more male behaviours. We know that aromatase is expressed in the brain, where it converts testosterone to estrogen.
3. The brains of both males *and* females express aromatase! Aromatase may be active during perinatal development, providing a source of neuroestrogens.
4. The estrogen detected in the ELISA may be a reflection of ovarian estrogens that have travelled to the brain rather than a measure of the brain's ability to make its own neuroestrogen.
5. Any value between 90-100pg/ml.

Resource Four
Model Answers

Answers

6. The concentration of estrogen may decrease over time for two reasons:

    - The brain cells may be starting to die after 8h, and so there are less cells making neuroestrogen.
    - If the measurements are reflecting the concentration of ovarian estrogens that reached the brain rather than the concentration of neuroestrogens synthesised by the brain itself, the decrease could be due to the fact that there is no ovarian supply of estrogens after the brain is dissected.

Resource Five Model Answers

Answers

1. Estrogen levels are low but slowly increasing across metestrus and diestrus. Estrogen levels peak at proestrus, in line with an acceleration of GnRH pulses and a surge in LH secretion, increasing the animal's sexual receptivity. Following this, estrogen levels sharply decrease, leading to low but slightly fluctuating levels in estrus. The cycle then starts again with low levels at metestrus.
2. Estradiol benzoate (EB) agonises both membrane receptors and nuclear receptors. However, paired with G-15 it can only activate membrane isoforms of ERα, like ERα-36. This means that although GPER1 is inhibited (by G-15), there are still some estrogen receptors active that can facilitate lordosis behaviours. G-1 only activates GPER1, and paired with G-15 GPER1 is silenced. Therefore, there is essentially no estrogen receptor activation resulting in a lower lordosis quotient.
3. The gonads are the major source of estrogens and androgens in the body. Removing them means that you can properly assess the contributions of any steroid treatment, without confusing them with the effects of gonadal steroids.
4. Behavioural experiment: resident intruder test.
   Animal treatments: control, G-1, G-1 + G-15 (extra points if you also suggested to use EB and EB + G-15!). OR you could use GPER1 knockout mice.
5. There is no right answer for this, just your opinion! Use the resource as well as any other knowledge or experiences you have to justify your answer.

Resource Six
Model Answers

Answers

1. Personalised treatments will allow us to tailor medications and treatments to the individual, giving them a better quality of life. For example, having personalised treatments could reduce side effects of less targeted medicine, as well as allowing people to find the treatment that works for them in a much quicker timeframe. However, we are currently held back from utilising and administering personalised treatments because we are still studying the sexual dimorphisms that contribute to differences in disease.
2. Animal behavioural studies must be carried out quickly, over as few times as possible, to minimise animal suffering.
3. By using ERα- and ERβ-specific agonists and antagonists, such as PPT (ERα) agonist, DPN (ERβ agonist) and tamoxifen or ICI-182,780 (ERa and ERβ antagonists).
4. The maze would have a '+' shape and be elevated off the floor. Two of the arms would be sheltered and walled up (closed arms) and two of the arms would have no walls and no shelter (open). Over the course of the test, you would time how long the animal spends in the open arms vs the closed arms. Time spent in the open arms indicates less anxiety, but time spent in the closed arms denotes anxiety. If the ratio of time spent in either location is greater in the closed arms, the animal is said to be more anxious.
5. Again, there is no right answer for this, just your opinion! Use the resource as well as any other knowledge or experiences you have to justify your answer.

Final Reflection Activity Further Guidance

Deep Dive

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Introduction

- A suitable discussion of the background and the reasons for doing this study.
- E.g. sexual dimorphisms contribute to the epidemiology of many psychological disorders, including depression. Depression has been related to estrogen exposure in the brain, but it is not known which receptor estrogen acts through to exert depressive effects.

Methods

- 6-9 animals should be used.
- A brief explanation of how the forced swim test is set up, e.g. the animal is dropped into a container of water that it can't stand up in. The time the animal spends swimming and trying to climb the container (indicative of less depressive behaviour) is recorded against the time the animal spent immobile (indicative of more depressive behaviour).
- Another experiment you could have chosen to do is a test for aggression, since it is common for depressed males to also be aggressive. Therefore, an explanation of how the resident-intruder test is set up.

Results

- You should describe the graph provided, e.g. WT male mice showed significantly less immobility in the early part of the test compared to the late part of the test. In contrast, GPER1 knockout male mice showed high levels of immobility times in both the early and late parts of the test, with no significant difference between either part of the test.
- If you also did a behavioural test for aggression, you would expect that GPER1 knockout male mice are more aggressive than WT male mice as a result of more depression (you could insinuate this from the fact that the provided graph shows a large amount of time being immobile, and thus the animals are more depressed).

Discussion

- The results indicate that GPER1 knockout male mice are more depressed than WT male mice, due to greater immobility times in the forced swim test and greater aggression in the resident intruder test.
- This indicates there could be a therapeutic potential for GPER1 in male depression, but we also need a better understanding of the role of ERα and ERβ first.

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