Mechanisms of anaesthesia – from molecular targets to neuronal networks

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Propofol

Potentiates inhibitory \( \text{GABA}_A \) receptors

Dexmedetomidine

Activates inhibitory \( \alpha_{2A} \) receptors
Knock-in mice can help determine the relevance of anaesthetic targets & pathways

The β3N265M Knock-in mouse is insensitive to propofol
Anaesthetics cause no structural changes at their binding sites


A propofol binding site on mammalian GABA_A receptors identified by photolabeling

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Propofol is the most important intravenous general anesthetic in current clinical use. It acts by potentiating GABA_A (\gamma-aminobutyric acid type A) receptors, but where it binds to this receptor is not known and has been a matter of some debate. We synthesized a new propofol analog photolabeling reagent whose biological activity is very similar to that of propofol. We confirmed that this reagent labeled known propofol binding sites in human serum albumin that have been identified using X-ray crystallography. Using a combination of protiated and deuterated versions of the reagent to label mammalian receptors in intact membranes, we identified a new binding site for propofol in GABA_A receptors consisting of both \beta_3 homopentamers and \alpha_1\beta_3 heteropentamers. The binding site is located within the \beta subunit at the interface between the transmembrane domains and the extracellular domain and lies close to known determinants of anesthetic sensitivity in the transmembrane segments TM1 and TM2.
Photolabelling with $o$-propofol diazirine

$Para$-propofol diazirine

$Para$-propofol dimethyl diazirine

$Meta$-propofol diazirine

$Ortho$-propofol diazirine

Photolabelling with $o$-propofol diazirine

Properties of ortho-propofol diazirine

Labelling human serum albumin

Bhattacharya et al. JBC 275, 38731 (2000)

Only a single amino acid is labelled in GABA<sub>A</sub> receptors – Histidine 267
Identification of the binding site

The propofol binding pocket only exists in the open state.

Anaesthetic binding to pre-existing states can explain kinetics

\[ \alpha_1 \beta_2 \gamma_2 \]

\[ \text{Unbound} \rightarrow \text{Bound 1} \rightarrow \text{Bound 2} \]

\[ \text{Closed 2} \rightarrow \text{Open 2} \]

GABA
Anaesthetic binding to pre-existing states can explain kinetics.

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How do anaesthetics act at the network level?

Neuronal ensembles sufficient for recovery sleep and the sedative actions of $\alpha_2$ adrenergic agonists

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Do sedatives engage natural sleep pathways? It is usually assumed that anesthetic-induced sedation and loss of righting reflex (LORR) arise by influencing the same circuitry to lesser or greater extents. For the $\alpha_2$ adrenergic receptor agonist dexmedetomidine, we found that sedation and LORR were in fact distinct states, requiring different brain areas: the preoptic hypothalamic area and locus coeruleus (LC), respectively. Selective knockdown of $\alpha_2A$ adrenergic receptors from the LC abolished dexmedetomidine-induced LORR, but not sedation. Instead, we found that dexmedetomidine-induced sedation resembled the deep recovery sleep that follows sleep deprivation. We used TetTag pharmacogenetics in mice to functionally mark neurons activated in the preoptic hypothalamus during dexmedetomidine-induced sedation or recovery sleep. The neuronal ensembles could then be selectively reactivated. In both cases, non-rapid eye movement sleep, with the accompanying drop in body temperature, was recapitulated. Thus, $\alpha_2$ adrenergic receptor–induced sedation and recovery sleep share hypothalamic circuitry sufficient for producing these behavioral states.

The ascending arousal system in the brain stem, hypothalamus and basal forebrain keeps the neocortex alert & aroused.

Activation of the sleep-promoting systems in the hypothalamus and basal forebrain silences these nuclei and promotes sleep.
cFOS is upregulated during sedation and deep sleep

cFOS is upregulated during sedation and deep sleep

TetTagging allows excited neurons to be tagged

Two viruses are injected which co-transfect the neurons

The first drives expression of the Tet Activator through a cfos promotor

In the presence of doxycycline, expression of hM₃D₉ is blocked

In the absence of doxycycline, the Tet Activator drives expression of hM₃D₉

The hM₃D₉ receptor can be subsequently selectively activated by CNO

TetTagging allows excited neurons to be tagged

AAV injection

4 weeks on doxycycline – TetTag system locked

OFF Dox

ON Dox

AAV transfection period, no hM₃D₉ expression

Dex or RS

CNO injection

Recovery sleep

Recovery sleep

Recovery sleep

Reactivation of excited neurons recapitulates behaviour

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Reactivation of excited neurons recapitulates behaviour

Dexmedetomidine sedation

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Role of GABA release in dexmedetomidine sedation

Reactivation of excited neurons recapitulates hypothermia

Reactivation of excited neurons recapitulates hypothermia

Summary and conclusions

- Anaesthetics act by binding to pre-formed cavities that exist in some conformational states of the protein, but not others.
- Propofol acts mainly on the GABA$_A$ chloride channel and binds to a site at the interface between the extracellular and transmembrane domains.
- Dexmedetomidine induces a state closely resembling natural sleep.
- The sedation by dexmedetomidine requires activation of a small group of neurons in the pre-optic hypothalamus.
- The same neurons, or at least an overlapping ensemble, are responsible for dexmedetomidine-induced hypothermia.
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