

Physiology Lab: Neuromuscular Junction Experiment in Frog

The neuromuscular junction of vertebrates has been intensely studied as a model of general synaptic function because its size and accessibility are greater than synapses within the central nervous system. This synapse is a critical point in communication between the neural and muscular systems.

Preparation of Frog Experimental Model

1. Remove the brain by cutting of the upper jaw from the caudal border of the tympanic membrane. Then destruct the spinal cord by pithing. This procedure will make the frog insensitive to pain and destroy the reflexes.
2. Keep the frog muscle moist at all times by dripping frog Ringer's solution on it.
3. Fix the frog to the dissecting plate from four limbs.
4. Place the pithed frog in a dissecting tray and cut through the skin completely around the leg in the thigh. Pull the skin downward and off the leg.
5. Dissociate the muscles at the femoral part of the leg and isolate the sciatic nerve and femoral artery. The nerve appears as thick and white structure while the femoral artery is black and thinner.
6. On one leg separate the femoral vein and femoral artery from leg and tie vein from downside, and artery from upper site. Cut the whole leg from calf without damaging the sciatic nerve. This leg will be totally isolated from circulatory system
7. Inject 0.5 ml of rocuronium bromide to the dorsal lymph sac of the frog and wait for 15-20 minutes. Rocuronium Bromide is a competitive AchR antagonist, which is used in modern anaesthesia as muscle relaxant.
8. Stimulate sciatic nerve and gastrocnemius muscle on both legs with a voltage over maximal stimulus.
9. Observe the responses.

Questions

1. Is there a response in both legs with indirect stimulation?
2. Is there a response in both legs with direct stimulation? And is there a difference of the response between two legs?