TITLE: MICROHETEROGENEITY OF THE Ca^{+2} ATPase IN SKELETAL MUSCLE FROM PIGS WITH MALIGNANT HYPERTHERMIA (MH).

AUTHORS: D. Mullikin-Kilpatrick, B.S., D.M. Ekenbarger, M.S., G. Welch, M.D., K. Townsend and J.L. Leonard, Ph.D.
AFFILIATION: Dept. of Anesthesiology, Univ. of Mass. Med. School. Worcester, MA 01655.

MH is an inherited disorder induced by halothane, and is associated with increased cytosolic Ca^{+2} levels in the skeletal muscle of affected individuals. Alterations in Ca^{+2} release from the sarcoplasmic reticulum (SR), mediated by the ryanodine sensitive Ca^{+2} release channel, and/or Ca^{+2} uptake, catalyzed by the SR Ca^{+2} ATPase, may account for the elevated cytosolic Ca^{+2} observed in MH skeletal muscle. To determine if structural differences in the Ca^{+2} ATPase result in hypersensitivity to denaturation and thereby a decrease in Ca^{+2} uptake, we compared the enzyme protein in normal (N) and MH pig muscle by chemical cleavage, limited proteolysis and by thermal inactivation. Northern analysis was used to examine the abundance of mRNA for the Ca^{+2} ATPase using a cDNA probe (450 bp) to rabbit fast-twitch skeletal muscle. Peptide fragments were generated using V8 protease, cyanogen bromide and N-chlorosuccinimide by the method of Cleveland et al[1] and separated on 12.5% PAGE gels. Ca^{+2} ATPase activity was determined by measuring the release of $^{32}P_i$ from γ-^{32}P labeled ATP. Peptide maps of the Ca^{+2} ATPase from N and MH showed different banding patterns, with 4 to 6 unique fragments. Differences in protein stability were also observed; after 2 min at 55°C, N muscle lost 59 $\pm$ 6% (mean $\pm$ SE, n=9) of the Ca^{+2} ATPase activity, while MH muscle lost only 40.3 $\pm$ 8% (mean $\pm$ SE, n=9) ($p<0.05$ N vs. MH) of the activity. These differences were observed in both homozygous and heterozygous animals suggesting that they were not related to strain. In total RNA preparations, we found equal quantities of the 3.7kb Ca^{+2} ATPase message in N and MH. Purification of polyadenylated mRNA by oligo dT selection showed that the amount of poly A^+ Ca^{+2} ATPase mRNA in the MH was only 14% of that in normal ($p<0.01$) indicating a defect in polyadenylation. Control blots showed no differences in the quantity of ß-actin mRNA in both total and poly A^+ mRNA from N and MH pigs. These data show microheterogeneity in the amino acid sequence of the Ca^{+2} ATPase and an altered degree of polyadenylation of the mRNA for Ca^{+2} ATPase in the skeletal muscle of MH pigs. The defect in polyadenylation may indicate mutation(s) at the 3'end of the gene for the Ca^{+2} ATPase. These differences in may provide useful markers for MH-susceptibility.

1. J Biol Chem 252:1102-1106, 1977

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TITLE: CUTANEOUS ANESTHESIA: EFFECTS OF PROPOFOL ON NON-NOXIOUS RESPONSES OF SPINAL DORSAL HORN NEURONS IN CATS

AUTHORS: H. Uchida, M.D., J.G. Collins, Ph.D., K. Kishikawa, M.D., H. Iwasaki, M.D.
AFFILIATION: Dept. of Anes., Yale University School of Medicine, New Haven, CT 06510

Spinal dorsal horn neurons play an important role in the sensory processing of afferent information. General anesthetics suppress noxiously evoked activity but less attention has been focused on their ability to alter activity of spinal dorsal horn neurons elicited by low intensity receptive field (RF) stimulation. We have recently reported pentobarbital suppression of activity elicited by low intensity stimuli.[1] Propofol, 2,6-diisopropylphenol, an I.V. anesthetic agent that provides rapid induction and emergence, has no structural relation to any other intravenous anesthetic. The purpose of this study was to examine, in intact, awake, drug-free animals, the effects of systemically administered propofol on the response properties of spinal dorsal horn neurons to low intensity stimulation of their peripheral RFs.

This protocol was approved by the Yale Animal Care and Use Committee. Extracellular recordings of single spinal dorsal horn neurons were made in physiologically intact, awake, drug-free cats (each cell served as its own control). The RF area that was sensitive to light touch was mapped before and after drug administration. Neuronal responses to brushing and pinching of the RFs were also determined before and after I.V. drug administration.

Eighteen low threshold neurons were studied. Propofol anesthesia (7.5-10.0 mg/kg) decreased by at least 20% the RF size of eleven neurons. Five RFs were reduced by 70 percent of the baseline value. The rapid and extremely smooth emergence from propofol anesthesia made it possible to observe complete recovery. When recovery was studied, most RF sizes returned to baseline as the animals awoke. The RF of three neurons was larger than baseline (> 120% of control) as the animal awoke. In addition to reducing RF size, propofol also reduced neural response to brushing (n=15) and to non-noxious pinch. Those responses also returned to baseline values as the animals awoke.

The use of physiologically intact, awake, drug-free animals permits us to study drug effects on neuronal systems that are functioning normally. Three measures of low intensity sensory input were observed to be depressed during propofol anesthesia. Taken together the reductions in response indicated that, for many cells, not only is the RF made smaller by propofol but the sensitivity within the remaining RF area is also reduced. De Jong[2] described, in acute experiments, a halothane induced reduction in RF area as "cutaneous anesthesia." We have reported that pentobarbital also reduced RF area.[1] Thus, reduced response to low threshold, as well as noxiously evoked afferent input at the spinal level has been demonstrated for several general anesthetics. Although general anesthesia is often thought of as a supraspinal phenomenon, reduced sensitivity to tactile afferent input within the spinal cord may be part of the mechanism by which anesthesia, the loss of all sensation, is achieved. If so, future studies of anesthetic effects on spinal dorsal horn neurons, as well as a better understanding of possible anesthetic effects on sensory processing by primary afferents, may provide insight into some of the mechanisms responsible for the production of anesthesia.

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1. Brain Res in press, 1990.
2. Exper. Neurol. 20:352-358, 1968.