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13. ABSTRACT (Maximum 200 words) We have studied the effects of elevated pressures of inert and narcotic gases on calcium-dependent functions and cytosolic calcium levels in human blood platelets, marine sponge cells and cultured human SK-N-SH neuroblastoma cells using the intracellular calcium indicator fura-2 AM. We found that many of the effects of narcotic gases and of pressure (He) could be explained by their influence on stimulated free cytosolic Ca^{2+} levels. The neuroblastoma cells provided much useful information. He pressure (18-36 ATA) potentiated carbachol-stimulated increases in $[Ca^{2+}]_i$ whereas Ar and N_2 did not. N_2O had the opposite effect and blocked the pressure-induced potentiation. Surprisingly, Xe had no effect despite being as potent an anesthetic as N_2O . We conclude that some of the effects of HPNS may be due to increased Ca^{2+} levels in neurons since- 1. this is compatible with its excitatory nature 2. the effects was opposed by narcotic gases 3. the effect was reversible when cells were compressed and decompressed before testing 4. the effect occurred at operational pressures. We further conclude that, given the different responses to N_2O and Xe, they cannot be ascribed to nonspecific anesthetic effects.					
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FINAL TECHNICAL REPORT

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For the past six years we have been investigating the relationship between Inert Gas Narcosis (IGN), High Pressure Neurological Syndrome (HPNS) and general anesthesia (GA) as they relate to cell membrane function, particularly regarding their effects on cellular activities involving calcium. Our initial studies employed human blood platelets and we found that platelet aggregation that was initiated by agents through mechanisms requiring extracellular Ca^{2+} were inhibited by moderate pressures (18-36 ATA) of either He or N_2 , whereas platelet functions that did not require an extracellular source of Ca^{2+} (phorbol-induced aggregation and shape change) were not affected by pressure of either gas (Ref #1). Further studies revealed that elevated pressures (4 ATA) of the anesthetic gas nitrous oxide (N_2O) also inhibited platelet aggregation whereas the noble gas xenon (Xe) actually potentiated aggregation, despite being at least as potent an anesthetic (Ref #2). These results suggested:

- a) That the responses could not be attributed to a non-specific effect of anesthetics given the difference between N_2O and Xe and
- b) That the different responses to He and Xe could be a manifestation of the opposite effects of pressure per se and a narcotic gas, even though the directions of change were not as expected.

Subsequent experiments of calcium-induced aggregation of marine sponge cells yielded essentially the same pattern, suggesting to us that the effects could be mediated via calcium-dependent, receptor-mediated aggregation involving bridging proteins (Ref# 2).

Since we were interested in the effects of pressure and anesthetics on intracellular Ca^{2+} levels, studies were conducted using the intracellular Ca^{2+} indicator fura-2 AM and ADP-stimulated human platelets. A pressure vessel was custom-built to fit the optical pathway of a Hitachi F4010 spectrofluorimeter. NO inhibited the ADP-induced rise in cytosolic free Ca^{2+} ($[\text{Ca}^{2+}]_i$) whereas Xe had no effect, further strengthening our suspicion that IGN was not simply a form of anesthesia (Ref #3).

At this point, our studies shifted to a cultured neuronal cell type, the human neuroblastoma cell line SK-N-SH. This cell is rich in muscarinic receptors of the M3 type (present throughout the CNS) and responds to muscarinic agonists such as carbachol with a marked increase in $[\text{Ca}^{2+}]_i$.

We found (Refs #4 & 5) that-

- i. He pressure (18 and 46 ATA) caused a 30% increase in carbachol-stimulated increase in $[Ca^{2+}]_i$.
- ii. Neither N_2 nor argon (Ar) at the same pressures increased $[Ca^{2+}]_i$; there was in fact a non significant decrease.
- iii. The effect of pressure was reversible. Cells compressed and decompressed slowly responded to carbachol like cells that had not been compressed at all.

Unpublished recent studies have shown that N_2O inhibited the carbachol-induced increase in $[Ca^{2+}]_i$ and blocked the pressure-induced potentiation of it. We have established several criteria for a cell model of IGN/HPNS which this preparation meets. To our knowledge, it is the only one currently to do so. The criteria are-

1. Responses should be compatible with the excitatory nature of HPNS.
2. The response to pressure (i.e. He) should be counteracted by anesthetic and narcotic gases.
3. The effect of pressure should be reversible.
4. THE Effects should be detectable at pressures encountered operationally by divers.

In conclusion, we feel that at least some of the manifestations of HPNS may be mediated by pressure-induced effects on cell calcium and that some, but not all, anesthetics work in part through opposite effects on cell calcium.

In a related study we have investigated the effects of ultra-high hydrostatic pressures (kilobar range) on the ligand and calcium-binding proteins albumin and fibrinogen and on the binding of carbachol to the enzyme acetylcholinesterase using Fourier-transform Infrared spectroscopy. Briefly we found that the presence of a ligand significantly altered the response of the protein to pressure. In general, the ligand-bound form was more resistant to pressure distortion (Refs 6-8).

PUBLICATIONS

1. Philp RB. Pharmacological studies on the mechanism of pressure inhibition of human platelet aggregation. *Aviat Space Environ Med* 61: 333-337, 1990
2. Philp RB, Arora P, Forsberg K, McIver DJ. Effects of pressure and gaseous anesthetics on the aggregation of human blood platelets and marine sponge cells: similarities in responses. *Comp Biochem Physiol C* 101: 541-545, 1992
3. Philp RB, Arora P, McIver DJ. Effects of gaseous anesthetics and ultrashort and short-acting barbiturates on human blood platelet free cytosolic calcium: relevance to their effects on platelet aggregation. *Can J Physiol Pharmacol* 70: 1161-1166, 1992
4. Philp RB, McIver DJ, Arora P. Effects of elevated pressures of inert gases on cytosolic free Ca^{2+} of human platelets stimulated with ADP. *Cell calcium* 14: 525-529, 1993
5. Philp RB, Kalogeros G, McIver DJ, Dixon SJ. Effects of elevated pressures of inert gases on cytosolic free Ca^{2+} of cultured human neuroblastoma cells stimulated with carbachol: relevance to high pressure neurological syndrome. *Cell calcium* 15: 117-121, 1994
6. Philp RB, McIver DJ, Wong PTT. Pressure distortion of an artificial membrane and the effect of ligand/protein binding. *Biochim Biophys Acta* 1021: 91-95, 1990
7. Kalogeros G, Wong PTT, Lecelle S, McIver, DJ, Philp RB. *Undersea Hyperbaric Med* 21: 1-7, 1994
8. Kalogeros G, Wong PTT, Philp RB. Comparison of responses to high pressure of albumin and fibrinogen in the presence and absence of calcium: a Fourier Transform infrared spectroscopic study. *Chem Phys Lett* in press

PUBLISHED CONFERENCE PROCEEDINGS

1. Philp RB. Effects of pressure and narcotic gases on aggregation of human blood platelets and marine sponge cells. *Proc. IInd Internat Meeting of Pressure Biology group, Toulon, 1990*
2. Philp, RB. effects of elevated pressures of inert narcotic gases on basal and stimulated free cytosolic calcium levels of human platelets. *Proc IIIrd Internat Meeting of Pressure Biology group, Duke U, 1992*

PUBLISHED ABSTRACTS

1. Philp RB. On the mechanism of He and N₂ pressure induced inhibition of platelet aggregation. Undersea Biomed Res 15(Suppl): 9, 1988
2. Philp RB, McIver DJ, Wong PTT. The effects of very high hydrostatic pressures and ligand binding on lipid-protein bonding in a model membrane. Undersea Biomed Res 16(Suppl): 85, 1989
3. Philp RB. Effects of elevated pressures of inert and narcotic gases on basal and stimulated free cytosolic calcium. Undersea Biomed Res 19(Suppl): 134, 1992
4. Kalogeros G, Philp RB. The effects of increased pressures of helium and the narcotic gases nitrogen and argon on calcium uptake by activated neuroblastoma cells. Undersea Hyperbaric med 20(Suppl): 75, 1993

PATENTS

None pending or applied for.

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