FINAL REPORT

for the Project

Neural Connectivity and Immunocytochemical Studies of Anatomical Sites Related to Nauseogenic and Emetic Reflexes

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INTRODUCTION

Motion sickness is an unpleasant, bothersome side effect of exposure to passive motion, but it seldom has important detrimental effects on the health of individuals. This malady typically it is of brief duration and the conditions that produce it often can be avoided. When the conditions producing motion sickness are unavoidable, the resulting "sickness" dissipates with continued exposure. In such cases individuals are said to have "adapted" to the sickness-inducing conditions.

However, when it is unavoidable, motion sickness can have significant short-term effects on the performance of individuals who are seriously affected. Because the susceptibility of individuals to motion sickness cannot be predicted with precision, some individuals can be seriously affected if they are required to work in an environment that produces motion sickness. Severe sickness in individuals of unknown susceptibility can become important if sickness arises when required tasks demand complicated, accurate performance. The occurrence of space motion sickness during reentry from space flight is one possible case of this type. Important human activities are required during launch and landing of the Space Shuttle, precisely the times skilled activity is required and when "space sickness" can occur.

There is considerable debate about the equivalence of emetic mechanisms involved in motion sickness produced in ground-based studies, "space sickness," and emetic responses in medical conditions (i.e., in chemotherapy). Ground-based studies of motion sickness provide certain benefits over flight studies. With this method studies can be conducted at considerable cost savings, the necessary control conditions can be included with experimental rigor, and the appropriate number of subjects can be used to address the experimental questions. Because there are numerous similarities between motion sickness and space sickness, it appears that better knowledge of motion sickness could significantly benefit the understanding and future study of space sickness. In addition, better understanding of emetic mechanisms in motion sickness may provide insights to mechanisms that are important to general medical conditions that involve emesis.

The studies conducted in this research project examined several aspects of neuroanatomical structures and neurochemical processes related to motion sickness in animal models. A principle objective of these studies was to investigate neurochemical changes in the central nervous system that are related to motion sickness with the objective of defining neural mechanisms that are important in this malady.

SUMMARY OF FINDINGS

For purposes of exposition, the studies and research findings have been classified into five categories. Detailed discussion of findings is presented in published papers that are provided in the Appendix.

A. Immunoreactivity in the Brainstem

Immunocytochemical techniques were employed to examine the distribution and ultrastructural appearance of glial fibrillary protein (GFAP), glutamine synthetase (GS), glutamic acid decarboxylase (GAD) and substance P (SP) reactivity in the area postrema (AP) and several other brainstem locations. These experiments were conducted to investigate a possible role of GABA and compounds associated with the metabolic cycle of GABA in emesis.

Strong immunoreactivity for GFAP was demonstrated in the ependymal cells lining the surface of the AP and in astroglial cells through out the AP. Immunoreactivity to GS was observed in both ependymal cells and atroglial components of the AP. These findings suggest that the ependymal lining and astrocytes of the AP (and neighboring structures, i.e., the subpostrema) might comprise an important complex in the physiology of the emetic reflex.

Varying densities of SP-reactive terminals and fibers were identified in the AP and adjacent structures. Immunoreactive terminals containing clear-core and dense-core vesicles which made symmetric or asymmetric synaptic contact with unlabelled dendrites were identifed using electron microscopy. It was suggested that GABA-ergic terminals might correspond to vagal afferent projections and that GAD/GABA and SP might be co-localized in the same terminal (D'Amelio *et al.*, 1988). Such a condtion could provide a putative mechanism for regulated release of transmitters in response to stimulation that produces motion sickness.

A role for the vagus nerve either in motion sickness or in adaptation to stimuli producing motion sickness has been suggested using immunocytochemistry techniques. The distribution pattern of GABA-ergic terminals in the area postrema, nucleus tractus solitarius, area sub-postrema, and gelatinous nucleus closely resembles that of vagal afferent projections (D'Amelio *et al.*, 1988). In addition, the

depletion of GAD immunoreactivity in these areas after electrical stimulation of the vagus nerve seems to confirm that at least part of the GABA-ergic activity shown here corresponds with vagal afferents. The additional demonstration of substance P immunoreactivity in this study implies there may be important neuromodulatory functions mediated by neuropeptides.

B. Vasopressin Effects

Vasopressin (AVP) is elevated in humans during reports of nausea and following vomiting (see Fox, 1992 for a review). Plasma AVP is dramatically elevated in cats following vomiting but the resting level of AVP in blood plasma does not differ among cats that are selected to be highly susceptible or very resistant to linear acceleration. On the other hand, AVP in cerebrospinal fluid (CSF) is not elevated following motion sickness, but resting levels of AVP in CSF are lower in animals that vomited during motion than in those animals which did not vomit (Fox *et al.*, 1987). The precise mechanism for the release of AVP during motions sickness could not be determined. Systemic injection of AVP at dosages calculated to produce levels equivalent to those observed following vomiting failed to produce vomiting or to influence the onset of vomiting in cats that were susceptible or resistant to to linear acceleration (Unpublished Data).

C. Lesion Studies of Area Postrema

Experiments using the lesion technique to examine the role of the area postrema showed that: (a) The area postrema is not involved in CTA that is produced by motion in rats (Sutton *et al.*, 1988); (b) Neither CTA nor vomiting are crucially dependent on the area postrema in either cats or squirrel monkeys (Fox, Corcoran & Brizzee, 1990). In combination with work by Borison and Borison (1986), these findings contributed to a reevaluation of the role of the area postrema in vomiting induced by motion (Daunton, 1990; Daunton *et al.*, 1987). several authors have now proposed theories which include several additional brainstem and/or circumventricual structures in the emetic response (see Fox, 1992 for references).

D. Role of the Vagus Nerve

A possible role for the vagus nerve in responses to motion is implied by results showing that the vagus nerve is crucial to CTA induced in rodents by exposure to motion (Fox & McKenna, 1988; Fox, Sutton & McKenna, 1988). Combined with other research, this finding shows that both vestibular and gastric neural systems contribute to the formation of CTA when motion is the stimulus. The specific mechanism by which gastric circuitry functions is unknown (Fox, Sutton, & McKenna, 1988), but we did provide evidence indicating that gastric afferents of the rat remain active for an extended period following brief physiological stimulation (Nijiima *et al.*, 1987; 1988).

E. CNS Structures Related to Adaptation to Microgravity

Autoradiographic analysis of benzodiazepine and muscarinic receptors was performed in various regions of the CNS in rats flown in the Soviet Biosatellite COSMOS 2044 (Hyde et al., 1992). Density of benzodiazepine ($GABA_A$) receptors did not differ among flight and control groups in any of the regions sampled. The density of muscarinic cholinergic receptors was significantly lower in the striatum of flight animals than in synchronous control animals. No significant differences were found among all groups in the other regions that were examined. These findings suggest that muscarinic cholinergic receptors in the striatum might be related to alterations in motor activity that are induced by chronic exposure to microgravity.

CONCLUSIONS

According to present understanding, the previous conception of the area postrema as the vomiting center in motion sickness was premature and incorrect. This conceptualization arose, in part, from interpretations of lesion experiments that were conducted before many of the diverse and sensitive techniques of neuroscience that are in common use today were available. Many researchers now argue that the emetic reflex is mediated via circuitry in several circumventricular and brainstem regions. Finding from this research support that position, but important work is required to provide understanding of the specific neural mechanisms of this response that is so important in disease and in travel by modern conveyances.

An overarching hypothesis that was developed during the course of this research is that motion sickness is a phenomenon that arises as only one of the outcomes of the more general effects of adaptation to unusual environments. Significant changes occur in peripheral muscle systems during spaceflight (D'Amelio & Daunton, 1992). Afferent feedback from these changes may initiate effects on neurotransmitter systems in the CNS that are related to the space adaptation syndrome.

Thus, motion sickness may develop when organisms are subjected to passive motion that results in atypical linear forces on the vestibular system. It is clear that an intact vestibular system is required for the production of this malady. It also appears, however, that the emetic reflex occurs only when adaptive responses in systems other than the emetic reflex (e.g., motor coordination, postural reflexes, etc.) are initiated. Consequently, it is reasonable to expect that many systems may be undergoing significant changes (i.e., adaptations) during periods when motion sickness occurs. In fact, motion sickness often can be avoided or ameliorated by adapting a behavioral strategy that prevents adaptation of motor systems. Thus, lying down or moving only in ways that avoids the maintenance of postural control may prevent the development of sickness. This observation suggests that motion sickness might occur as an unfortunate byproduct of normal processes through which the neuromuscular system adjusts to new, atypical environmental conditions. Although the specific mechanisms that may be involved in such processes are obscure at this time, discovery of the physiology and neural changes that underlie the process of adaptation may predict the mechanisms that elicit motion sickness.

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APPENDIX I.

RESEARCH PAPERS

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