6-OHDA to intrahypothalamic administration of norepinephrine or Poly I:Poly C, and a comparison of these responses with those of vehicle-treated animals. It was found that selective depletion of hypothalamic norpinephrine significantly reduced the Poly I:Poly C-induced fever. However, the fever induced by intrahypothalamic injections of norepinephrine was not affected by selective depletion of hypothalamic norepinephrine.

Thus, it appears that noradrenergic pathway occurs in the hypothalamus which mediates the febrile responses to Poly I:Poly C or IFN. In the present results, the febrile responses to Poly I:Poly C may be related to the endogenous release of norepinephrine from the rat's hypothalamus. Intrahypothalamic injection of Poly I:Poly C may have resulted in activation of hypothalamic noradrenergic receptors and thus cause febrile responses. The Poly I:Poly C-induced, but not the norepinephrine-induced, fever would be prevented by depletion of hypothalamic norepinephrine. Norepinephrine injected intrahypothalamically is believed to act on postsynaptic adrenergic receptors in the hypothalamus to induce fever. The results are supported by the findings of many investigators. For example, intrahypothalamic injection of norepinephrine or other adrenergic agonists caused febrile responses[12,13]. The norepinephrine-induced fever was antagonized by pretreatment of the rat's hypothalamus with adrenergic receptor antagonist[12]. Microiontophoretic application of norepinephrine reduced the activity of warm-sensitive units in the hypothalamus, whereas the discharge rate of cold-sensitive units in the rat's hypothalamus was elevated[14]. Recently, our pilot study also demonstrated that the Poly I:Poly C-induced fever was underpinned by central administration of adrenoreceptor blocking agents (Lin et al., unpublished data).

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Thermoadaptive influence on reactivity pattern of vasopressinergic neurons in the guinea pig.

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Summary. In cold-adapted guinea pigs, increased amounts of arginine-vasopressin (AVP) immunoreactive material could be visualized in neurons of the supraoptic and paraventricular nucleus, in fibers projecting to the neurohypophysis and in fiber terminals in the ventral lateral septum and in the amygdala. In warm-adapted animals the reactivity to AVP antiserum was poor in all neuronal structures examined. High AVP-immunoreactivity was accompanied by a reduced febrile response to bacterial pyrogen in cold-adapted guinea pigs.
Key words. AVP; immunohistochemistry; nucl. paraventricularis and supraopticus; ventral lateral septum; thermal adaptation; antipyresis; guinea pig.

In a previous study[1] we found that in guinea pigs peripheral release of AVP is dependent on ambient temperature. In animals born and reared at 22 °C the level of AVP in arterial plasma was 3.2 pg AVP/ml, and in 24-h urine samples 6.8 ng AVP/day. Adaptation to 5 °C caused a 2–3-fold increase of AVP concentration in blood plasma and an 8–10-fold increase in daily excreted amounts of AVP. Adaptation to 28 °C was accompanied by a 20–30% reduction of AVP in blood plasma and in 24-h urine, compared with values at 22 °C. The influence of these thermoadaptive changes in peripheral release of AVP on the water-balance in guinea pigs has already been described in detail[1].

A high release of AVP into different parts of the central nervous system has been postulated for other physiological situations as well. Increased amounts of vasopressinergic material could be demonstrated immunocytochemically in the septum and amygdala of guinea pigs at the

end of pregnancy, and in newborn animals. In both cases the febrile responses to injections of bacterial pyrogen were strongly reduced[2]. Considerable evidence supporting the existence of endogenous peptidergic antipyretic systems in the limbic system has been accumulated in recent studies[3-5]. The role of AVP as an antipyretic agent was demonstrated by the complete suppression of fever during intraseptal microinfusions of AVP in physiological concentrations in the guinea pig[6].

In order to investigate which pools of AVP neurons are activated during cold-adaptation, and whether vasopressinergic pathways to the neural lobe of the pituitary, to the ventral septal area and to the amygdala are concurrently stimulated by a cold environment we performed the following study.

Materials and methods

Guinea pigs born and reared in our animal house at 22 °C under a 12-h day and 12-h night cycle were used in this study, which was performed in early winter. When the animals had reached a weight of 300–400 g they were put into individual metabolic cages which were placed in climatic chambers at 5 °C or 28 °C. Body weight, food and water consumption, volume of urine and weight of feces were controlled in each animal every morning. At the end of the third week of adaptation to 5 °C or 28 °C six cold-adapted and six warm-adapted male animals were killed and their brains were processed for immunochemistry by methods described previously[7].

The amounts of AVP-immunoreactive material were compared in the brains of cold- or warm-adapted animals in neurons of the supraoptic nucleus (SON) and of the paraventricular nucleus (PVN), in fibers passing the median eminence and projecting to the neurohypophysis and in fibers and nerve terminals in the ventral lateral septa and in the amygdala.

The febrile reactions to intramuscular injections of bacterial endotoxin (*E. coli*, 20 µg/kg) were tested in groups of cold-adapted and warm-adapted guinea pigs. The fever responses were plotted as colonic temperature-time curves for 6 h after the pyrogen injection. The integrated area under the temperature-time curves, the fever index, was expressed in °C · h (for 6 h). The colonic temperature was measured every 30 min by inserting a thin plastic coated thermocouple 6 cm beyond the anus. This procedure did not cause excitement or any apparent alternation in the body temperature of the animals.

The fever indices of cold- and warm-adapted guinea pigs were compared by Student's t-test.

Results

Remarkably high amounts of AVP-immunoreactive material were observed in perikarya of the SON in cold-adapted animals; high reactivity to AVP antiserum could also be demonstrated in fibers passing the internal zone of the median eminence and projecting to the neurohypophysis in this experimental group (fig. 1 a, b).

Much less AVP-immunoreactivity could be visualized in SON-neurons of warm-adapted guinea pigs. In these animals hardly any AVP-fibers were found in the internal zone of the median eminence (fig. 1 c, d).

These results were in good correlation to increased peripheral release of AVP in cold-adapted guinea pigs and reduced release of AVP in warm-adapted animals[1]. The number of AVP-immunoreactive perikarya and the intensity of immunoreactivity in the PVN were increased during cold-adaptation. Numerous AVP neurons were visualized in the medial part of the PVN in cold-adapted guinea pigs. In the corresponding area of the warm-adapted animals only few AVP neurons could be demonstrated (fig. 2 a, d).

A large number of immunoreactive AVP-fibers and fiber terminals were revealed in the ventral lateral septa and in the amygdala of cold-adapted guinea pigs, while the immunoreactivity to AVP antiserum was very poor in the corresponding brain areas of the warm-adapted group (fig. 2 b, c, e, f).

The activation of AVP neurons in the medial area of the PVN and of AVP fiber terminals in the septum have been discussed as mechanisms in fever regulation, in fever suppression and in the development of tolerance to pyrogens[8,9]. Therefore we tested the effects of intramuscular injections of bacterial pyrogen on the febrile response of cold-adapted and warm-adapted guinea pigs.

The development of fever after application of bacterial endotoxin was not completely suppressed in cold-adapted animals, but was remarkably reduced in comparison to warm-adapted guinea pigs. The fever index was 4.25 °C · 6 h in the warm-adapted group and only 2.6 °C · 6 h in the cold-adapted animals (fig. 3).

Discussion

In contrast to the peripheral functions of AVP as an antidiuretic hormone and a vasoactive agent, the role of ascending vasopressinergic projections to different brain areas has not yet been completely elucidated[10]. It has been postulated that AVP acts as a neuromodulator in behavioural pathways and a consolidator of memory. From our previous studies we obtained both indirect and direct evidence for functional roles of AVP as an endogenous antipyretic and as an effector in the homeostatic control of fever. A complete suppression of fever after application of bacterial pyrogen could be demonstrated by microinfusion of exogenous AVP into the septum in concentrations of 9.6 µg/6 h up to 0.13 µg/6 h in the guinea pig[6]. Fever suppression by intraseptal AVP infusion has also been demonstrated in other species[5]. A special aim of this investigation was to answer the question whether stressor stimuli which increase the release of AVP into the circulation concurrently activate the AVP projections to the CNS. After osmotic stimulation,

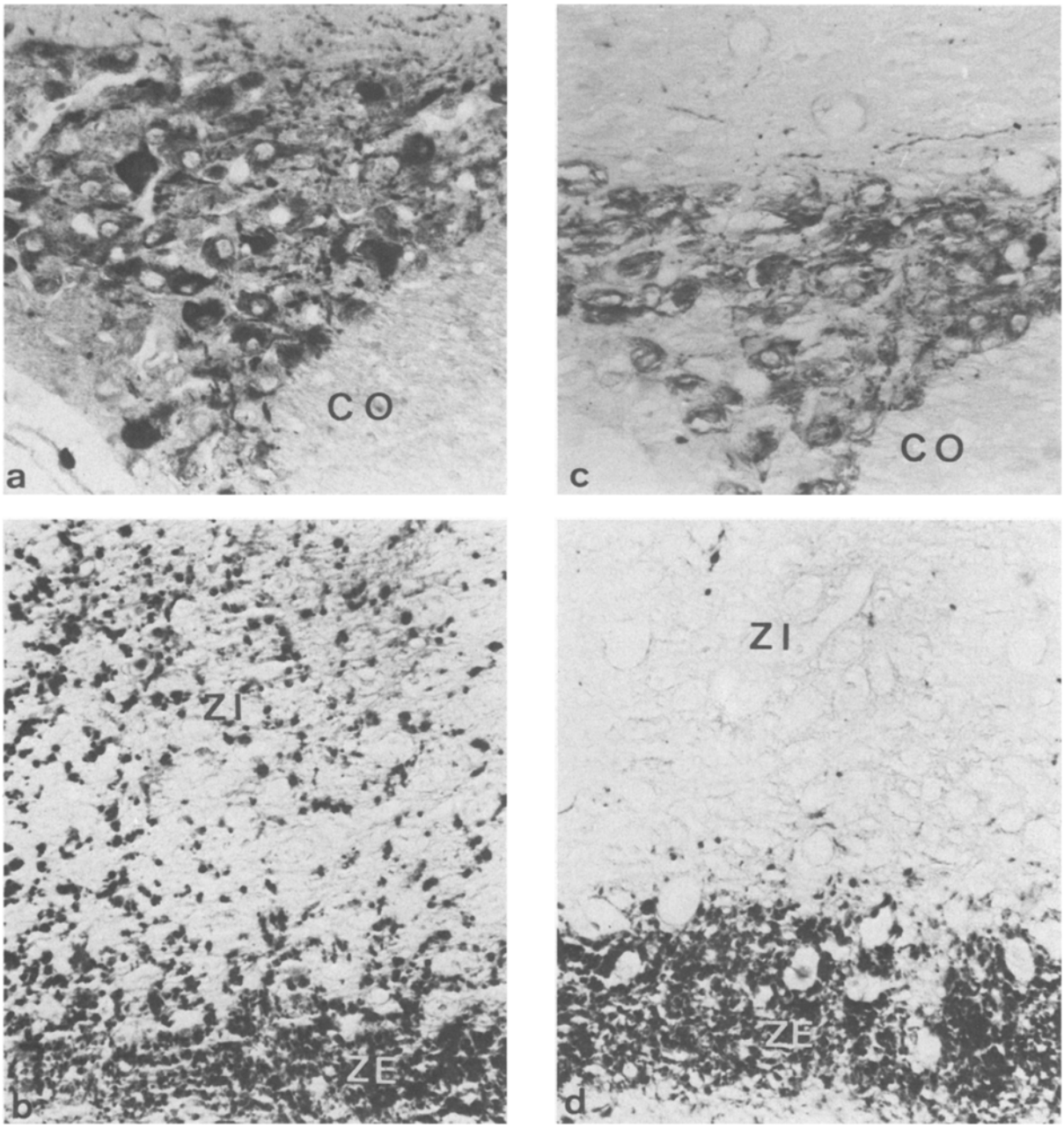

Figure 1. Frontal sections of cold-adapted (*a, b*) and warm-adapted (*c, d*) guinea pigs. *a, c*: SON; *b, d*: medial area of the median eminence; in the cold-adapted animal strongly AVP-reactive perikarya and fibers are prominent in the SON (*a*). Fibers in the internal zone (ZI) of the median eminence contain increased amounts of reactive material (*b*). In the warm-adapted animal the reactivity to AVP antiserum is reduced in the SON (*c*) and in the ZI of the median eminence (*d*); CO, optic chiasma; ZE, median eminence, external zone; *a–d*: × 350.

higher levels of AVP could be detected in blood plasma and cerebrospinal fluid in rats [11]. Chronic exposure to cold has been demonstrated to be a non-osmotic cause of enhanced AVP turnover in guinea pigs [1]. In the present study we documented increased amounts of AVP-immunoreactive material in different parts of the CNS during cold-adaptation. Thus there is evidence that stressors of non-osmotic origin can also activate central and peripheral release of AVP concurrently.

However, the extent of activation of release either to the CNS or to the peripheral circulation seems to differ in the case of osmotic or non-osmotic stimuli, being higher in the periphery in the first case and in the CNS in the second case. Moreover, the observed activation of AVP pathways to the areas of the limbic system was accompanied by a reduction of the febrile response after pyrogen application. According to the described effects of exogenous AVP as an effective antipyretic drug [6] we concluded

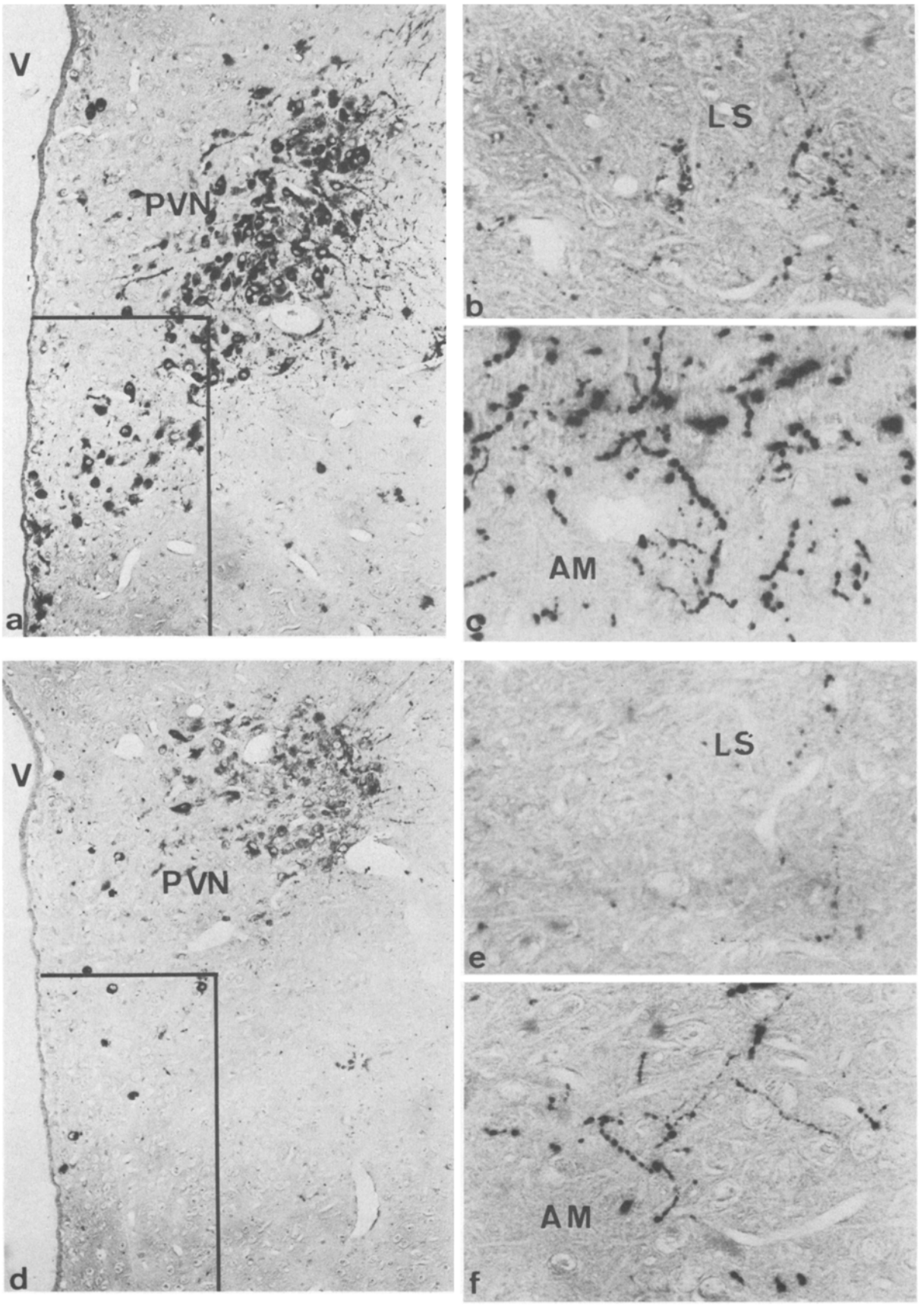

Figure 2. Frontal sections of cold-adapted (*a–c*) and warm-adapted (*d–f*) guinea pigs. PVN, paraventricular nucleus; LS, lateral septum; AM, amygdala; V, third ventricle; note the AVP-reactive neurons in the medial portion of the PVN in cold-adapted animals (marked areas); *a, d*: × 140; *b, c, e, f*: × 560.

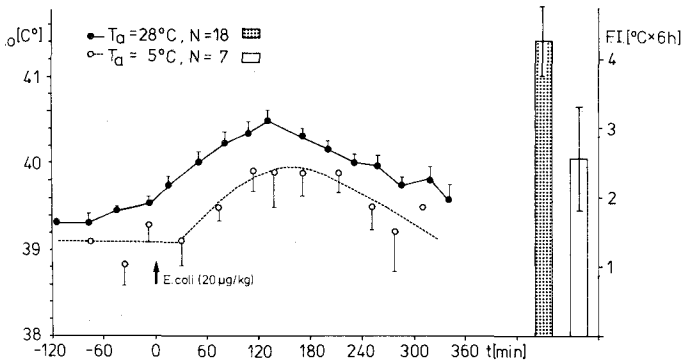

Figure 3. Comparison of fever responses to bacterial pyrogen (*E. coli*, 20 µg/kg i.m.) in groups of warm-adapted (black symbols, shaded column) and cold-adapted (white symbols and column) guinea pigs. The fever indices of cold- and warm-adapted animals differed significantly ($p < 0.05$).

that there is a causal relationship between stimulation of ascending vasopressinergic projections to the septum and fever reduction after pyrogen application during cold-adaptation of guinea pigs. The biological significance of enhanced AVP release in different stressful situations and the related antipyretic reaction has not been fully elucidated yet. The interactions between centrally released neuropeptides and autonomic functions merit further endocrinological, physiological and pharmacological studies.

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Gamma-aminobutyric acid uptake by rat kidney brush-border membrane vesicles

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Summary. Brush-border membrane vesicles (BBMV) from rat kidney cortex possessed two uptake systems for γ-aminobutyric acid (GABA), a high affinity system (Km = 10.9 µM) and a low affinity system (Km = 1203 µM). Both uptake systems were inhibited by p-hydroxymercuribenzoic acid and ouabain, and by the action of neuraminidase, whereas the GABA analogs nipecotic acid, β-alanine, 2,4-diaminobutyric acid and 4,5,6,7-tetrahydroisoxazolo-[4,5c]-pyridin-3-ol had no effect on the GABA uptake activity. The BBMV uptake systems were clearly different from the GABA transport systems present in brain tissue.
Key words. GABA; transport; kidney; brush-border membrane vesicles.

The existence of two γ-aminobutyric acid (GABA) uptake systems in brain tissue had been recognized for many years[1], but more recently, using a wide range of substrate concentrations and computer-assisted analysis, the authors demonstrated the presence of three GABA uptake systems in synaptosomes and brain slices[2,3]. Since the presence of a high affinity GABA transport system in brush-border membrane vesicles (BBMV) from rat kidney cortex had been reported[4], the current study was initiated, again with a wide range of substrate concentrations and computer-assisted analysis, to determine whether multiple uptake systems for GABA existed in kidney tissue. The identification of two uptake systems and a comparison of their properties with those of the brain tissue transport systems are described below.

Methods

Renal cortex was obtained from male Wistar rats (200–250 g b. wt), and brush-border membrane vesicles prepared from the tissue by the method of Goodyer et al.[4]. GABA uptake was measured as described previously[3]. In essence, the vesicle preparation was preincubated at 25 °C for 15 min, ^{3}H-GABA was added, and a further 10-min incubation was carried out. The vesicles were then recovered by Millipore filtration and counted by liquid scintillation spectrometry. Non-specific binding to