TABLE VI. *In Vitro* Formation of Inosine 5'-phosphate by Cell-Free Extracts of Ascites Cells.[a]

| Substrate | Tumor cell line | Nucleotide formed (mμmole per min per mg of protein) |
|---|---|---|
| Inosine-8-$^{14}$C | Ehrlich Sensitive | 6.30 |
| Inosine-8-$^{14}$C | L1210/MP/MeMPR[b] | 0.55 |
| Inosine-8-$^{14}$C | L1210/MP | 0.45 |

[a] The reaction conditions were the same as those described in Table I.

[b] This subline is doubly resistant to 6-thiopurine and methylthioinosine.

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## Lack of Directional Selectivity of Cassaine in Excitation of the Medullary Emetic Chemoreceptor Trigger Zone from Blood and Cerebrospinal Fluid in Cats*† (32710)

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The chemoreceptor trigger zone (CTZ), situated in area postrema of the medulla oblongata, has been proposed as the receptor site for the emetic action of a variety of drugs (1–3). Two major experimental bases for this proposal are first, that the emetic response to intravenous injection of a given agent is abolished by surgical ablation of the CTZ (1,2), and second, that local administration of the agent, as by injection into the cerebrospinal fluid (CSF), is a potent means of evoking emesis (3). In the case of cardiac glycosides, however, it was found in the cat that this class of drugs is ineffective in eliciting emesis through the intracerebroventricular route even though these agents satisfy the first criterion for an emetic stimulant action at the CTZ (4). It was suggested therefore

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that the emetic receptors in the area postrema are accessible to stimulation by the cardiac glycosides from the vascular side only of the blood–CSF barrier (4).

Such directional selectivity of the glycosides has also been described for the inhibition *in vitro* of sodium transport in toad bladder (5), frog skin (6), squid axon (7), and other tissues (8). In each instance, the drugs are effective only when applied to the side of sodium extrusion. The erythrophleum alkaloids share with the cardiac glycosides certain major pharmacological properties as well as the ability to inhibit Na–K-activated adenosine triphosphatase and Na transport (8,9). However, in contrast to the glycosides, erythrophleine has been found to be as effective on the mucosal as on the serosal surface of toad bladder (5). This lack of directional selectivity in the action of erythrophleum alkaloids *in vitro* provides the rationale for the present comparison of the emetic effectiveness of the member alkaloid cassaine given by intravenous and by intracerebroventricular injection. Drug–receptor interaction at the chemoreceptor trigger zone was examined further by use of known emetic drugs, namely the cardiac glycoside deslanoside and the opiate apomorphine, in selected combinations of treatment with cassaine.

*Methods.* All drug injections were made in unanesthetized cats. The animals were prepared with a permanently indwelling cannula in the lateral or third cerebral ventricle (10). Only those results obtained in animals with confirmed cannula placements are reported here. Cannulas were flushed with approximately 0.25 ml of sterile saline between drug tests. The intraventricular drug injections were given in a total volume of 0.20 ml (11). Intravenous injections were made either by direct puncture of the cephalic vein or through a permanently indwelling catheter placed in the jugular vein. Cassaine sulfate and apomorphine hydrochloride were obtained in powder form and were dissolved in sterile saline. Deslanoside was taken from commercial ampules (Sandoz). The drugs were tested routinely for their emetic effectiveness shortly after the animals were fed a milk meal. Actual expulsion of vomitus was accepted as the sole criterion of a positive emetic response. A period of at least 5 days was allowed for recovery between emetic tests. Ablation of the chemoreceptor trigger zone in area postrema of the medulla oblongata was accomplished by gentle thermal cauterization.

TABLE I. Emetic Responses to Cassaine Sulfate Administered Intravenously (iv) and Intracerebroventricularly (icv) Before and After Ablation of the Chemoreceptor Trigger Zone (CTZ).

| | CTZ intact | | CTZ ablated |
|---|---|---|---|
| Dose | Incidence | Latency mean (range) (min) | Incidence |
| iv (mg/kg) | | | |
| 0.25 | 5/6 | 18 (5–60) | — |
| 0.50 | 6/6 | 6 (3–11) | 0/4 |
| icv (mg total) | | | |
| .002 | 0/2 | — | — |
| .004 | 1/4 | 4 | — |
| .010 | 3/4 | 7 (5– 8) | — |
| .020 | 4/4 | 6 (2–10) | 0/3 |

*Results. Effect of CTZ ablation on the emetic activity of cassaine.* The consistently effective *intravenous* emetic dose of cassaine sulfate in nonoperated animals was found to be 0.50 mg/kg (Table I). This dose was not lethal in any instance. Emesis was produced in all of six cats with an average latency of 6 min. Chronic ablation of the CTZ eliminated the emetic response in all of four operated cats. The consistently effective *intraventricular* emetic dose of cassaine in nonoperated animals was found to be 0.20 mg (total), which is approximately 1/50 to 1/100 of the dose required intravenously. There is no overlap in the two ranges of effective emetic doses. It is noteworthy that the average latency of emesis was the same for both routes of administration. The CTZ ablation abolished the emetic effect of intraventricular cassaine in all of three operated cats. Normal animals responded to cassaine with from one to four emetic episodes and showed recovery within an hour. In contrast, the emetic effect of intravenous deslanoside along with its sequelae of anorexia and malaise lasts into the next day and longer.

*Tachyphylaxis and receptor blockade.* To

TABLE II. Emetic Tachyphylaxis Produced by Repeated Intracerebroventricular (icv) Injection of Cassaine Sulfate and Its Influence upon the Emetic Response to a Subsequent Intravenous (iv) Injection of the Drug.

| Route | icv (0.02 mg) | | | iv (0.50 mg/kg) | |
|---|---|---|---|---|---|
| Injection time (min) | Initial | 30 | 60 | 70 | 120 |
| No. vomited | 3/3 | — | 0/3 | — | 2/2 |
| No. tested | 8/8 | 4/8 | 0/8 | 0/2 | — |

examine the hypothesis that identical receptors are activated by cassaine from both the blood and CSF sides of the CTZ, an attempt was made to produce drug "occupation" of the receptors through one route and then to test for an emetic response by the other route. Tachyphylaxis was demonstrated in two sets of experiments in which repeated intraventricular injections of cassaine were administered (Table II). In one set with three trials a second dose (0.02 mg) given 60 min after the first failed to produce vomiting. However, in the other set with eight trials, four responses were obtained when the second dose was administered only 30 min after the first, but the cats were refractory in all trials to a third dose given 30 min after the second. Thus it is evident that about 60 min are required for full development of tachyphylaxis and, presumably, for adequate occupancy of receptors after intraventricular injection of cassaine. When cassaine was given intravenously 60 min after the second of two intraventricular doses (0.04 mg total), the expected response to the intravenous injection was neither prevented nor delayed. However, the emetic response to intravenous cassaine was prevented when the drug was injected 10 min following pretreatment with three intraventricular doses (0.06 mg total) over a 60-min period. To exclude the possibility of cassaine being absorbed and acting systemically after intraventricular injection, the same dosage schedule as above that produced blockade of the emetic response was given wholly by the intravenous route. No blockade of emesis resulted from this regimen in all of three animals used although the average response latency to the final test injection was prolonged from 6 to 25 min. It is thus apparent that receptor occupancy by cassaine is characterized by weak binding which is consonant with the early symptomatic recovery observed after effective treatment with the drug.

These experiments demonstrate that both intraventricular and intravenous cassaine act upon identical receptors to produce vomiting, and that the drug reaches these receptors from either side of the blood–CSF barrier.

*Interaction between different emetic drugs.* Table III summarizes studies on the interaction of cassaine with other emetics previously demonstrated to act at the CTZ. Pretreatment with intraventricular cassaine in the same regimen which blocked emesis to intravenous cassaine (self blockade) failed to influence the emetic response to consistently effective doses of deslanoside (0.16 mg/kg, intravenously) and apomorphine (0.2 mg total, intraventricularly). These results indicate only that drug–receptor interactions involving deslanoside and apomorphine differ from those involved in the action of cassaine, but no light is shed on whether the same receptor elements participate in the different emetic responses. The converse approach also was used, namely to pretreat with one of the other agents and then to test with cassaine. Owing to the long-lasting effect of deslanoside, presumably due to persistent receptor occupation, cassaine (0.02 mg total) was administered intraventricularly at 24 hours after intravenous injection of the glycoside (0.16 mg/kg). Of four cats so tested, two were blocked against cassaine inasmuch as emesis failed

TABLE III. Summary of Emetic Interaction of Cassaine Sulfate with Deslanoside and Apomorphine Hydrochloride Given in a Variety of Sequences by Intracerebroventricular (icv) and Intravenous (iv) Injection.

| Pretreatment | Challenge | Emetic blockade |
|---|---|---|
| Cassaine, icv | Cassaine, iv | Yes |
| | Deslanoside, iv | No |
| | Apomorphine, icv | No |
| Deslanoside, iv | Cassaine, icv | Yes |
| Apomorphine, icv | Cassaine, icv | No |

to occur within an hour after injection of the drug, although the remaining cats vomited after normal latencies. It was determined that apomorphine (0.2 mg total) given intraventricularly produced emetic refractoriness to itself when a second dose was injected at 30 min after the first. Nonetheless, intraventricular cassaine elicited vomiting without prolongation of latency in two cats treated 30 min earlier with intraventricular apomorphine. These experiments provide evidence for the possibility that cassaine acts upon the same emetic receptors as does deslanoside, but not upon those acted upon by apomorphine.

*Discussion.* The present experiments demonstrate that, although erythrophleum alkaloids and cardiac glycosides both produce emesis in cats by stimulation of the chemoreceptor trigger zone in area postrema, cassaine is equally effective from both the blood and CSF whereas the glycosides have been shown to be effective only from the vascular side of the CTZ. Since cassaine administered intraventricularly can block the emetic response to itself on systemic administration, it is evident that the same receptors are reached by the drug through either route. The short duration of effect due to cassaine, as contrasted to that described for deslanoside, suggests that the binding characteristics of these two drugs are quite different. We have obtained some evidence that deslanoside can block the response to cassaine even after a 24-hour interval, which indicates that the drugs may be acting at the same receptor elements. This should not be surprising in light of the many similarities of action already found between these two classes of drugs. Apomorphine is a reliable emetic drug when given by the intraventricular route, even though it does not consistently cause emesis in cats when injected intravenously (3). However no interaction of apomorphine with cassaine was found in the present study thereby indicating separate mechanisms of receptor activation for apomorphine and cassaine.

Our results suggest that the difference in directional characteristics of the erythrophleum alkaloids and cardiac glycosides could be due to a difference in their penetrabilities. Unfortunately, although the same kind of directional effect was found for sodium transport inhibition in toad bladder, frog skin, squid axon, cat choroid plexus, and other tissues, no direct study of the penetrability of such tissues by either of these classes of compounds is known. Recent electron-microscopic studies of area postrema in the rabbit (12) and cat (13) describe perivascular spaces around the capillaries in this region. These spaces are bounded by a basement membrane which projects into the nervous tissue. We could explain our observations if drugs were able to reach the "emetic receptor" only by way of these perivascular spaces. Thus, systemically administered cardiac glycosides and erythrophleum alkaloids could gain ready access to the perivascular space through the permeable capillary endothelium. Drugs administered intraventricularly, however, must pass through the ependyma and, because the perivascular space is not continuous with the extracellular fluid space, they have to penetrate the basement membrane in order to gain access to the perivascular space. Since several studies indicate that the ependyma is freely permeable (14,15), it follows that the basement membrane constitutes the crucial barrier to drug penetration from the CSF into the pericapillary spaces of the area postrema. Accordingly, we may postulate that this basement membrane is permeable to erythrophleum alkaloids but not to cardiac glycosides.

There is at present no indication for a relationship between emesis and sodium transport. In fact, it is interesting that tetrodotoxin, which is believed to reduce membrane permeability to sodium in squid axon (16), has no effect on sodium transport in frog skin and toad bladder (17). However, like the glycoside ouabain, tetrodotoxin is effective only when added to the external surface of squid axon (16), and, like ouabain, it causes emesis in cats when given systemically but is ineffective on intraventricular administration (2). These findings argue against the concept of a link between Na transport inhibition and directional selectivity of drug action.

*Summary.* Cassaine sulfate produced emesis in cats when given by intracerebroventricular (0.02 mg in 0.20 ml) and intravenous (0.5 mg/kg) injection with an average latency

of 6 min in either case. The emesis elicited through both routes of administration was eliminated after chronic ablation of the chemoreceptor trigger zone in the area postrema of the medulla oblongata. The emetic response to intravenous cassaine was blocked by pretreatment with intraventricular cassaine. Intravenous deslanoside only partially blocked the emetic activity of intraventricular cassaine. These results indicate that the emetic receptors of the chemoreceptor trigger zone are accessible to cassaine from the blood as well as from the cerebrospinal fluid, and that identical receptor elements are stimulated by the drug through both routes of administration. This lack of directional selectivity of cassaine stands in contrast to the known selectivity of the cardiac glycosides.

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## Effect of Experimental Portacaval Shunt on Hepatic Drug Metabolizing Enzymes* (32711)

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Previous experiments in our laboratory have demonstrated that end-to-side portacaval shunts in rats produce hepatic injury. Diversion of the portal blood from the liver leads to hepatic siderosis (1), increased DNA synthesis, and ultrastructural changes in hepatocytes (2). However, in view of the large reserve functional capacity of the liver, these changes by themselves should not necessarily be considered evidence of a hepatic functional deficit. Since clinical portacaval shunts are often followed by an encephalopathy (3), which may result from interference with the detoxifying ability of the liver in addition to the bypass of ammonia from the liver, we thought it interesting to study this facet of liver function by assaying the effects of the experimental portacaval shunts on hepatic detoxifying enzymes.

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*Material and Methods.* End-to-side porta-