There was often another spot which also seemed to be more intense after a rapid i.v. injection than after s.c. administration or slow i.v. infusion of 5HT in rabbit. This spot also showed more activity in rat stomach preparation after HCl hydrolysis than before it. This spot probably was due to the 5HT O-sulphate ester giving R_f-values corresponding to those of Chadwick and Wilkinson.[10]

A spot corresponding to the O-glucuronide of 5HT was obtained also from the urine collected from a carcinoid patient during and after severe flush attacks. Before paper chromatography the urine was precipitated by 40 vols of acetone, and the active material dissolved from the sediment into pyridine. The spot was not present in the urine of a healthy human subject after ingestion of 2 mg/kg of 5HT or 1/2 kg of peeled bananas.

The results give support to the opinion that the phenolic conjugation with sulphonic and sulphuric acids is an emergency route for the elimination of 5HT. The faster the amine enters the circulation the more of the conjugate is formed. On the other hand, there is an indication that the most intense ester formation occurs after oral administration. It is also probable that a part of the 5HT O-glucuronide and O-sulphate esters is further oxidised to the corresponding 5HIAA conjugates.

The results will be published in detail elsewhere.

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Actions of cocaine and tyramine on the uptake and release of H^3-norepinephrine in the heart

(*Received* 8 *June* 1961)

PREVIOUS work in this laboratory has shown that circulating H^3-catecholamines are taken up and bound in the heart, spleen and adrenal gland.[1, 2] We have also observed that pre-treatment of cats with cocaine[3] or tyramine[4] markedly reduced the concentration of H^3-norepinephrine in heart and other tissues. This effect might be a consequence of an inhibition of the uptake of the circulating hormone or a release of the bound H^3-catecholamine, or both. To enable us to distinguish between these actions, these compounds were given before or after the administration of H^3-norepinephrine. If the drug interfered with the uptake, it would decrease the H^3-norepinephrine only if given before the administration of the catecholamine. If, after the injection of H^3-norepinephrine, when the catecholamine is bound in the tissue, the level of amine in the tissue is lowered by the drug, the latter releases the bound hormone.

Male rats (Sprague–Dawley) weighing from 160–180 g were given 10 μc of DL-7-H^3-norepinephrine (20 mc/mg) per 100 g by injection into the tail vein and the animals were killed 2 hr later by decapitation. The hearts were homogenized with 12 ml of perchloric acid (0·4 N). After centrifugation, the supernatant solution was assayed for H^3-norepinephrine.[2] Cocaine HCl, 10 mg/kg, was given

10 min before or 10 min after the H^3-catecholamine and tyramine HCl, 15 mg/kg, was injected 10 min before or 20 min after the H^3-norepinephrine. All drugs were given intravenously. In the combined treatment, cocaine was given 10 min and tyramine 20 min after H^3-norepinephrine.

There was a striking decrease in the amount of H^3-norepinephrine taken up by the heart when the animals were given cocaine before the H^3-catecholamine (Table 1). No effect was observed when the cocaine was given after the H^3-norepinephrine. Tyramine reduced the amount of the H^3-norepinephrine found in the heart when it was administered either before or after the H^3-catecholamine. These observations indicate that cocaine blocks the uptake of H^3-norepinephrine, while tyramine releases the bound hormone. Tyramine might also prevent the uptake of the circulating H^3-norepinephrine.

TABLE 1. EFFECT OF COCAINE AND TYRAMINE ON THE UPTAKE AND RELEASE OF H^3-NOREPINEPHRINE IN RAT HEART

Treatment	H^3-norepinephrine mμc/g heart (±s.e.m.)
Control	161 ± 12
Cocaine before H^3-norepinephrine	43 ± 2
Cocaine after H^3-norepinephrine	160 ± 20
Tyramine before H^3-norepinephrine	22 ± 1·3
Tyramine after H^3-norepinephrine	56 ± 7
Cocaine + tyramine after H^3-norepinephrine	44 ± 6

Each rat received 10 μc of H^3-norepinephrine per 100 g, given intravenously, and the hearts were assayed 2 hr later. Details of drug treatment are described in the text.

Tyramine showed no pressor action in animals whose norepinephrine stores were depleted with reserpine,[5] but after an infusion of norepinephrine its actions were restored.[6] On the basis of these observations, Burn and Rand[6] postulated that the actions of tyramine are mediated through the release of norepinephrine. Cocaine has been shown to antagonize the pressor actions of tyramine[7] and increase those of catecholamines.[8] We have previously shown that cocaine potentiates the actions of norepinephrine by preventing the inactivation by binding and elevating the blood levels of the hormone.[3] The results reported here show that tyramine releases H^3-norepinephrine from the heart. Since cocaine blocks the actions of tyramine and tyramine exerts its effects by liberating norepinephrine, cocaine would be expected to prevent the releasing actions of tyramine. This possibility was examined in the following experiment. Cocaine was given 10 min and tyramine 20 min after H^3-norepinephrine. The results in Table 1 show that cocaine had no effect on the release of H^3-norepinephrine by tyramine in the rat heart. These findings disagree with those of Lockett and Eakins,[9] who found that cocaine blocks the releasing action of tyramine. Schümann, using isolated granules from the adrenal medulla, found that cocaine did not block release of catecholamines by tyramine.[10]

If the actions of tyramine are mediated through the release of norepinephrine, it should be expected that cocaine would enhance the effects of the catecholamine released by tyramine. But cocaine prevents the pressor actions of tyramine and yet does not block the release of norepinephrine by the sympathomimetic amine.

It can be concluded from our experiments that cocaine inhibits the uptake of circulating H^3-norepinephrine, while tyramine releases the bound H^3-catecholamine. Cocaine does not block the releasing action of tyramine.

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Effect of a phosphatido-peptide fraction of intestinal tissue on the intestinal absorption of a quaternary ammonium compound*

(*Received* 8 *June* 1961)

PREVIOUS studies have shown that although quaternary ammonium compounds are incompletely absorbed from the intestine, there is always a portion that is absorbed and this portion is absorbed fairly rapidly following administration.[1] It has been postulated that the absorbed portion of the quaternary ammonium compound, which exists as a cation at all physiological levels of pH, is transferred across the gut wall as a neutral complex by virtue of combination with an endogenous anion.[2] Since a number of phosphorus-containing compounds have been implicated as possible carriers in the transport of endogenous inorganic and organic cations across biological membranes,[3, 4] the role of such phosphorus-containing anions in the transport of exogenous organic cations is now under investigation.

A phosphorus-containing fraction, first isolated from brain and designated "phosphatido-peptide" by Folch,[5] has been found in intestine, as well as in heart, liver, lung, spleen, pancreas and kidney.[6] The phosphatido-peptide represents a fifth category of tissue phosphorus compounds, since it cannot be classified as acid-soluble phosphorus, free phosphatides, nucleic acids or phosphoprotein, as usually defined.[7] The phosphatido-peptide fraction is constituted by inositol, phosphate, esterified fatty acids, sphingosine or sphingosine-like material and amino acids combined in polypeptide chains.[5, 6] This report presents preliminary evidence that the phosphatido-peptide fraction from intestinal tissue may be involved in the intestinal absorption of a mono-quaternary ammonium drug, benzomethamine (N-diethylaminoethyl-N′-methylbenzilamide methobromide).

METHODS

The material used in these studies was a relatively crude fraction of rat small intestine, prepared by the method of Huggins and Cohn,[6] including removal of (1) acid-soluble components with trichloroacetic acid, (2) phospholipides with alcohol-ether, (3) nucleic acid with 10% sodium chloride, and (4) extraction of the crude fraction from the remaining phosphoprotein by chloroform-methanol-HCl. This acidified chloroform-methanol extract was evaporated to near dryness *in vacuo* and the oily residue was taken up and suspended in Krebs-Henseleit phosphate buffer, pH 7·4, for use in the absorption studies. This suspension was regarded and used as the phosphatido-peptide fraction.

Non-fasted male rats of the Sprague–Dawley strain, weighing 180–210 g, were used. The degree of intestinal absorption was determined, *in vivo*, using either a single-loop preparation or a double-loop preparation, as previously described.[2] Double-loop preparations were used for controls, the loop positions of the control and experimental solutions to be compared being alternated in successive rats. Three kinds of control solutions were used: (1) benzomethamine dissolved in buffer, as a standard; (2) benzomethamine dissolved in a buffered solution of reagent blank of the extraction procedure, as a negative control; (3) benzomethamine dissolved in a buffered solution of pure albumin carried through the extraction procedure, as a pseudo-tissue control. A constant volume of 0·5 ml/loop

* This work was supported in part by a Research Grant (B-1966) from the Institute of Neurological Diseases and Blindness, U.S. Public Health Service, Bethesda, Maryland.