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In vitro release of endogenous histamine, together with noradrenaline and 5-hydroxytryptamine, from slices of mouse cerebral hemispheres

Evidence supporting the assertion that biogenic amines function as neurotransmitters in the central nervous system is much greater for noradrenaline, dopamine, 5-hydroxytryptamine (5-HT) and acetylcholine (Bloom & Giarman, 1968; Andén, Carlsson & Häggendal, 1969) than for histamine (Green, 1964; 1970). One of the characteristics to be expected of a neurohumour is that it should be stored in a readily-releasable form in neurons, and be released into the synaptic cleft in response to stimulation and depolarization of the nerve terminal membrane. Noradrenaline, dopamine and 5-HT have been shown to be stored in neurons of the central nervous system, and the release of these amines and acetylcholine has been demonstrated both *in vivo* and *in vitro*. Depolarization has been induced in brain slices by either electrical stimulation or increased concentrations of K^+ in the incubation medium (for review see Katz & Chase, 1970). Using the latter technique, we have recorded the release of endogenous histamine, noradrenaline and 5-HT from slices of mouse cerebral hemispheres.

The results now presented were obtained from a study primarily designed to observe the effects of different drugs on the metabolism of indoles in slices of mouse hemispheres and on the release of monoamines *in vitro*, as influenced by changes in the $[K^+]$ in the incubation medium. Histamine was determined in only a limited number of experiments.

Normal white female mice (NMRI), 18–24 g, were decapitated and the hemispheres (minus corpus striatum) were dissected, sliced and weighed. Six hemispheres were incubated for 40 min in 5 ml Krebs-Henseleit solution (Krebs & Henseleit, 1932) (equilibrated with 5% CO_2 in 95% O_2) which was modified by adding ethylene diamine tetra-acetate (EDTA), 15 μg/ml, and ascorbic acid, 20 μg/ml. Drugs were added for the purpose of the indole study and the $[K^+]$ was varied as indicated in Fig. 1. After incubation, the 'Krebs' was decanted and the slices blotted dry. In each experiment, two groups of six mice provided 24 hemispheres. Six hemispheres from each group were incubated in 'normal $[K^+]$-Krebs' and six in 'high $[K^+]$-Krebs', all other parameters being identical. Subsequently, the 'normal $[K^+]$-Krebs' from both groups were combined, as were the appropriate slices: the 'high $[K^+]$-Krebs' were similarly combined as were the slices (for further details see Carlsson, 1970). The proteins in the 'Krebs' solutions and in the slices were precipitated with perchloric

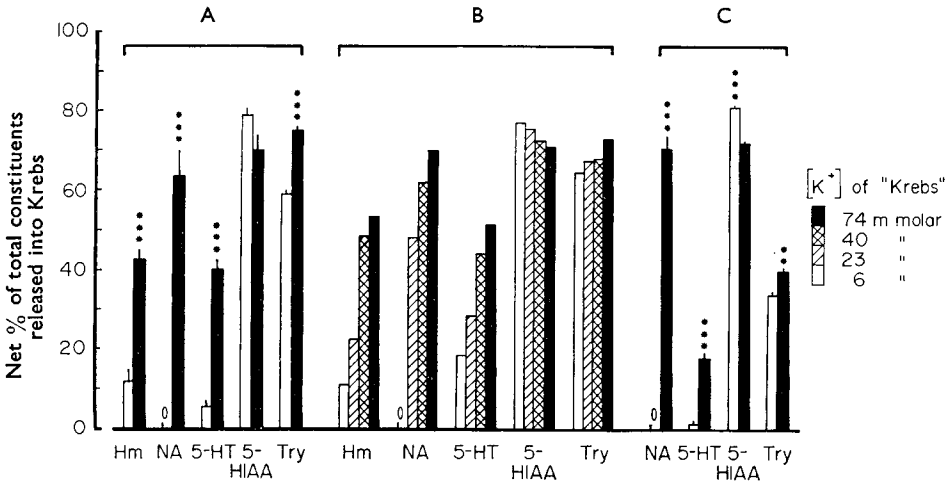

FIG. 1. Effect of changes in the $[K^+]$ in the incubation medium on the release of endogenous histamine (Hm), noradrenaline; (NA), 5-hydroxytryptamine (5-HT), 5-hydroxyindole acetic acid (5-HIAA) and tryptophan (Try) from slices of mouse cerebral hemispheres *in vitro*.

A modified Krebs-Henseleit incubation medium was used to which drugs were added: (a) tryptophan, 20 μg/ml; and pargyline, 1–100 μg/ml. (b) tryptophan, 40 μg/ml; pargyline, 10 μg/ml; and chlorimipramine, 0·3 μg/ml. (c) no additions.

Different $[K^+]$ were used: 6 (normal); 23; 40; or 74 mM (high), equivalent reductions being made in the $[Na^+]$ from 143 mM (normal). Shown are means and s.e. of 8 values (a) and 3 values (c) (single values in (b), where each individual value is derived from 12 mouse hemispheres and expresses the net percentage release of the total content of the constituent (average of 37 ng/g (a) for Hm; 149 ng/g (a), 137 ng/g (c) for NA; 623 ng/g (a), 280 ng/g (c) for 5-HT; 506 ng/g (a), 614 ng/g (c) for 5-HIAA; and 189 μg/g (a), 13 μg/g (c) for Try. Significance between 'normal' and 'high $[K^+]$-Krebs' is estimated from an analysis of variance (p × q), followed by the student's *t*-test: $^{**}P<0{\cdot}01$; $^{***}P<0{\cdot}001$.

acid containing EDTA and $Na_2S_2O_5$. After centrifugation, the supernatants were further processed and the amines and related compounds were purified on Dowex 50W columns before their fluorimetric determination (for methods see Atack & Magnusson, 1970; Atack, 1971; Lindqvist, 1971; Bédard, Carlsson & Lindvqist, 1972). From the determinations of these compounds in the 'Krebs' and slices after incubation the net percentages released into the 'Krebs' were calculated.

Increasing the $[K^+]$ in the incubation medium induced a marked increase in the percentage releases of the amines. A much smaller increase in the release of tryptophan and a slight decrease in the release of 5-HIAA was observed (Fig. 1a, c). The extent of these changes in percentage releases appeared to be dependent on the magnitude of the increase in $[K^+]$ (Fig. 1b). No effect of different concentrations of pargyline (1, 2·5, 40 and 100 μg/ml—Fig. 1a) on the K^+-induced percentage releases of the constituents could be ascertained. Addition of tryptophan to the incubation medium markedly influenced the average total contents and percentage releases of 5-HT and tryptophan (compare Fig. 1a and 1c) but not of noradrenaline, and histamine is more likely to resemble noradrenaline.

Electrical stimulation induces an increase in the release *in vitro* of exogenous noradrenaline and 5-HT from brain slices (Baldessarini & Kopin, 1967; Chase, Katz & Kopin, 1969). Furthermore, the increase in release of glutamate, aspartate and GABA, amino-acids known to have neurophysiological activity (see Curtis & Johnston 1970), appears to be greater than for other amino-acids e.g. tyrosine (Bradford, 1970). Electrical stimulation has been shown to be relatively specific. The release of exogenous urea, inulin and 5-HIAA, all substances which are not concentrated in nerve terminals, is not increased (Chase & others, 1969). The quantitative release of amines and amino-acids was found to be similar, whether induced by electrical

stimulation or an increased [K^+], but the specificity of the latter method was not established (see above references). Our results show that increasing the [K^+] caused a marked increase in the release of endogenous histamine, 5-HT and noradrenaline, but not of the supposedly non-transmitters 5-HIAA and tryptophan.

The use of Dowex 50W columns for purifying biogenic amines (see Atack & Magnusson, 1970), has enabled us to measure changes in brain histamine levels in experiments conducted primarily to study the monoamines. Moreover, the normal value of 48 ng/g obtained for histamine in whole mouse brain using fluorimetric assay (Atack, 1971), can be compared with recent values of 60 ng/g obtained using organic solvent extraction and fluorimetric assay (Anton & Sayre, 1969), 60 ng/g by bioassay (Adam, H., personal communication, 1967 in Green, 1970) and 49 ng/g by an enzymatic-isotopic procedure (Taylor & Snyder, 1972).

A highly significant increase in the release of endogenous histamine from slices of mouse cerebral hemispheres *in vitro* was induced by raising the [K^+] in the extracellular medium. This increased release more closely resembled the changes in release of endogenous noradrenaline and 5-HT, than of tryptophan and 5-HIAA. In the case of the monoamines, which are stored within the neurons, this effect is probably due to depolarization of the nerve membranes. If histamine is similarly stored, then an analogous explanation of the observed histamine release is likely, and would provide further evidence that histamine may have a neurohumoural function in the central nervous system.

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