Sound-stimulated ^{14}C-glutamate release from the nucleus cochlearis[1]

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Summary. The exogenous release of glutamate has been successfully studied in the cochlear nucleus of guinea-pigs after physiological sound stimulation of the ear (frequency 2000–20,000 Hz at 100 dBA).

The concept of chemical transmission involves the release of transmitter substances from the presynaptic terminal. Hitherto, no significant release of a substance has been demonstrated in the central synapses after the use of physiological stimuli.

There is mounting evidence suggesting glutamic acid to be a transmitter of the primary afferent terminal in nervous acusticus. Its concentration is decreased in the cochlear nucleus after lesion of the auditory nerve[2]. Kainic acid injections (supposedly destroying neurons with glutamate receptors), result in degeneration of cells innvervated by the auditory nerve[3].

In this study we applied exogenous ^{14}C-glutamic acid to the exposed cochlear nucleus of the guinea pig. Washing the cochlear nucleus in saline, we were able to demonstrate its release upon sound stimulation of the ear.

Material and methods. Guinea-pigs of approximately 400 g were anaesthetized with urethan (1 ml/100 g of a 20% solution) and stabilized in a head-holding device. The skull was exposed, and the bone above cerebellum removed. The cerebellum was removed by suction with water vacuum; this left the cochlear nucleus visible to the eye. A special push-pull cannula (figure 1) was placed onto the surface of the nucleus and physiological medium (tris-buffered-saline-glucose) was applied via a peristaltic pump at a rate of 0.25 ml/min. Labelling was done in the physiological medium containing ~1 µCi/ml of either ^{14}C-glutamic acid, ^{3}H-aspartic acid on ^{3}H-valine, superfusing the cochlear nucleus for 20 min during sound stimulation with sine-waves tones of 2000–20,000 P./sec, continuous sweep, 2 sweeps/sec, sound level 100 dBA.

The surface of the nucleus was washed in medium for 25 min whereafter 0.1 mM p-chloromercuriphenyl-sulphonate (p-CMS), a reuptake inhibitor[4], was added for 5 min. Finally, sound stimulation was applied for 20 min during washing. Samples were collected at 1- or 2-min intervals throughout the experiment (see text to figure 2).

Controls: ^{3}H-valine with sound stimulation, ^{14}C-glutamate without sound stimulation, ^{14}C-glutamate with sound stimulation and the nervus acusticus cut off at the brain stem.

Results and discussion. Sound stimulated release is shown in table and figure 2. Following an initial wash-out period of ^{14}C-glutamate, presumably accumulated in superficial or extracellular parts of the cochlear nuclei, a plateau is obtained. The addition of the p-CMS causes some stimulation of the release which is, however, minor compared with the prominent increase caused by sound stimulation. In control experiments (table) the sound stimulation release of ^{14}C-glutamate is compared with that of ^{3}H-aspartate and ^{3}H-valine. Whereas there was no sound-stimulated release of ^{3}H-valine, some stimulated release was obtained, using ^{3}H-aspartic acid. This could either mean that a portion of the aspartic acid has been converted to glutamic acid, or that there are aspartatergic terminals present in the cochlear nucleus[2], or, which might be more likely, the aspartate is to some extent co-transported with glutamate. To clarify this, more experiments would be necessary.

The demonstration of ^{14}C-glutamic acid release from the cochlear nucleus after sound stimulation further strengthens the evidence for the presence of glutamatergic terminals in the primary afferent ending of the nervus acusticus,

Release of exogenously labelled substances from the cochlear nucleus upon sound stimulation

Compound	Stimulation (%)
^{14}C-glutamate	29 ± 12 (4)
^{3}H-aspartate	24 ± 12 (3)
^{3}H-valine	Not detectable

Percent stimulation was calculated as the extra amount of radioactivity released during the stimulation period in percent of the total radioactivity released during the same period. Values represent mean ± SD.

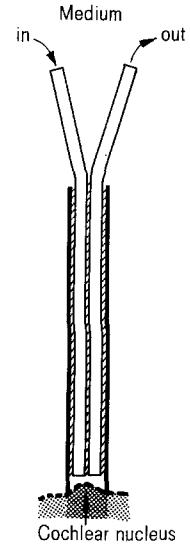

Fig. 1. The push-pull cannula which was applied to the cochlea surface. The diameter of the push-pull cannula is approximately 2.5 mm.

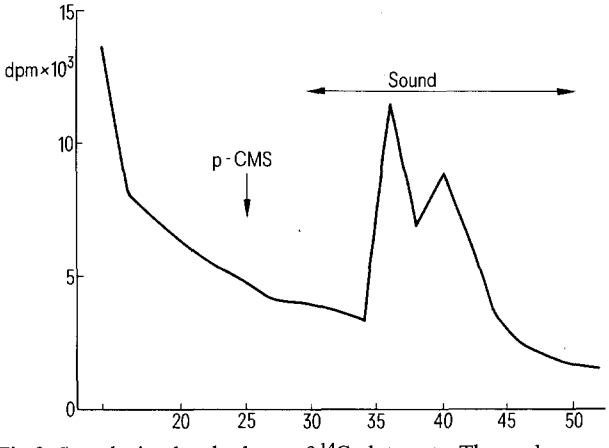

Fig. 2. Sound stimulated release of ^{14}C-glutamate. The nucleus was labelled for 20 min during sound stimulation. Washed for 25 min before p-CMS was applied. Sound stimulation was applied as indicated. Fractions of 0.25 ml were collected each min during p-CMS application, otherwise at 2-min intervals.

primarily in the anteroventricular cochlear nucleus (AVCN)[3-5]. Other transmitters discussed in this area of the brain are: acetyl choline, which has been located primarily to the granular layer of the cochleus nucleus (GCN)[6], and γ-aminobutyric acid, which has been located predominaltly to the AVCN[7]. Although our aim in this study has mainly been to develop a system where transmitter release could be demonstrated, given physiological stimuli, it is certainly of interest in future studies to localize more precisely those terminals releasing ^{3}H-glutamate, and to confirm that exogenous ^{3}H-glutamate could be used as a marker for the endogenous transmitter.

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Dopamine and cerebral cortical blood flow in the rat

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Summary. Dopamine topically applied to the cerebral cortex (1–20 µg/ml) or administered i.v. (0.5–64 µg/kg/min) has no effects on cerebral cortical blood flow in the rat.

Dopamine agonists induce complex central nervous effects[1-4]. The possible role of cerebral vascular receptors in those actions has been suggested, since nerve terminals containing dopamine have been demonstrated in cerebral arteries[5-7] and dopamine has been shown to modify blood flow in several organs[8]. Several authors have reported increases in cerebral blood flow following intravenous administration of dopamine agonists[9,10]. The effects of topical application of dopamine to cerebral blood vessels in vivo have not been evaluated however, and the possibility exists that the above mentioned effects of intravenous dopamine agonists might be mediated indirectly, particularly in view of the fact that dopamine constricts (rather than dilates) cerebral vessels in vitro[11]. To evaluate a possible direct cerebral vascular action of dopamine, this drug was topically applied to the exposed cerebral cortex while local blood flow was being measured at the same site by the hydrogen clearance technique. In another series of experiments, cerebral cortical blood flow (CoBF) was measured in the same way while dopamine was given intravenously.

98 albino rats were used. The animals were anesthetized by intraperitoneal administration of 1.5 g/kg urethane. A femoral artery was cannulated and the mean arterial pressure (MAP) was continuously monitored. The parietal cortex was exposed on one side and a platinum electrode (30 µm in diameter) was inserted 0.5 mm into the cortex in order to record the tissue hydrogen (H_2) concentration. H_2 was given by inhalation until a steady state concentration was attained in the tissue. The rate of tissue H_2 desaturation after interruption of the H_2 inhalation was used to calculate blood flow. Details of the technique can be found elsewhere[12,13]. Dopamine, dissolved in synthetic cerebrospinal fluid[14] at concentrations from 1–20 µg/ml was topically applied to the cortex surrounding the H_2 sensing electrode. In another set of experiments, dopamine was infused i.v. at rates from 0.5 to 64 µg/kg/min. The concentration of the solutions was adjusted to deliver the same infusion rate of fluid (6.8 µl/min) in all experimental groups. CoBF was measured immediately before and at 30 min intervals after the commencement of the dopamine application or infusion. In a number of animals, only 0.9% NaCl was infused, or artificial CSF was applied as a control.

Results and discussion. Topical application of dopamine to the cerebral cortex did not change CoBF significantly (table 1). During continuous intravenous infusions, a progressive increase in CoBF was observed for all concentrations of the drug as well as in animals infused with 0.9% NaCl alone. MAP showed a tendency to decrease during the infusion for all concentrations tested. It is concluded that dopamine has no effect on CoBF when administered topically or i.v. to rats.

The increase in CoBF during prolonged infusions, both in controls and in treated animals, is probably related to a slow dissipation of the anesthetic (which was given in a single injection at the beginning of the experiments) since CoBF has been shown to be related to the level of urethane

Table 1. Cortical blood flow (CoBF) before and after topical application of dopamine to the cerebral cortex

	Mean CoBF before application of test solution (± SE)	Mean change (± SE)* in CoBF after application of test solution	n
Synthetic CSF (no drug)	43.26 ± 13.52	0 ± 2.73	15
Dopamine 1 µg/ml	51.88 ± 5.71	5.50 ± 4.83	8
Dopamine 5 µg/ml	31.75 ± 2.74	2.17 ± 2.70	12
Dopamine 10 µg/ml	35.20 ± 6.74	3.50 ± 2.89	10
Dopamine 20 µg/ml	27.29 ± 2.22	2.07 ± 2.10	14

CoBF is given in ml/100 g/min. All differences were insignificant with the Student t-test for paired samples. * Mean ± SE of the difference between the first determination of CoBF after, and the last determination before, application of test solution. n = number of animals.