RELEASE OF ACETYLCHOLINE FROM THE BRAIN IN VIVO: SOME COMMENTS ON ESTIMATION METHODS AND THEIR APPLICATION

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Abstract. Techniques (push-pull cannula, cup, brain ventricles perfusion) allowing estimation of the amount of the ACh released in vivo from the brain are described. The main attention is paid to biochemical, physiological and morphological factors influencing the amount of ACh released and available for estimation. Conditions of experiments for each of these techniques are described in details. The amount of ACh released in different physiological states of several animal species is compared. Typical applications of all three methods are given.

INTRODUCTION

The aim of this review is to describe some of the methods used to determine the amount of neurotransmitters, specifically acetylcholine (ACh), released in the brain of anesthetized or freely moving animals. Pepeu in his recent review (71) has described some pharmacological aspects of this subject. This review will therefore concentrate mainly on the factors influencing the accuracy of these techniques and their applications.

FACTORS INFLUENCING THE RELEASE OF ACETYLCHOLINE

General rules for the determination of the amount of ACh released in vivo

It is generally accepted that the major mode of neuron communication in the brain is through the release of neurotransmitters. These che-
mical compounds are released from presynaptic nerve terminals following excitation, diffuse across the synaptic cleft and interact with postsynaptic receptors, thus producing either a depolarization or hyperpolarization of the postsynaptic membrane. Inactivation of the neurotransmitter may take place by: a) enzymatic hydrolysis of the neurotransmitter (e.g. ACh); b) reuptake by the cell from which it has been released (e.g. noradrenaline); c) uptake by glial cells (as is postulated e.g. for glutamic acid) or by all three simultaneously.

Inhibition of hydrolysing enzymes or blocking of uptake elevates the amount of neurotransmitter released into the synaptic cleft. As a result of diffusion the concentration of neurotransmitter also increases in the extracellular space in the ventricles, and on the surface of the brain.

Depending on the area of the brain the following techniques may be adopted to measure the amount of released neurotransmitter: a) push-pull cannula technique for subcortical structures; b) cup technique for the surface of the brain.

One of the most intensively investigated neurotransmitters is acetylcholine. It is released by cholinergic nerve terminals in constant multimolecular amounts: quanta (31, 45, 52). After depolarization of the postsynaptic membrane, ACh is hydrolysed by acetylcholinesterase (AChE) to choline and acetic acid. Some of the ACh, which is not hydrolysed may diffuse from the synaptic cleft into the extracellular space (53) as it is shown in Fig. 1. The final concentration of ACh in the extracellular space depends on the balance between released and hydrolysed neurotransmitter.

Application of the above mentioned techniques for estimation of the amount of released ACh is shown in Fig. 2.

As suggested by Katz and Miledi (53), a high concentration of ACh in the synaptic cleft following presynaptic stimulation of peripheral nerves partially inhibits AChE activity. Thus more ACh would appear in the extracellular space but the amount of choline available for uptake and ACh synthesis would decline. In this way the concentration of ACh in the synaptic cleft could regulate further synthesis and release of ACh. The amount of ACh diffusing from the synaptic cleft depends on several factors: a) the surface, area, and volume of the synaptic cleft; b) the temperature of the brain (it is especially important to warm the animal or the exposed tissue during acute experiments); c) presynaptic and postsynaptic membrane potentials; d) the concentration of ions in the synaptic cleft; e) AChE activity; f) the density of receptor sites on the postsynaptic membrane to which ACh may attached (53). If one wants to estimate the amount of ACh rele-
Fig. 1. Scheme illustrating diffusion of ACh in the extracellular space: A, active AChE; B, inhibited AChE; 1, ACh released into extracellular space; 2, synaptic vesicles containing ACh; 3, synaptic cleft; 4, postsynaptic density. Note the difference in ACh concentration (degree of stippling) in the extracellular space between A and B.

Fig. 2. Possibility of applying push-pull cannula and cup techniques for estimation of ACh released in vivo.
ased in vivo it is necessary to inhibit the activity of AChE. This is usually done by adding phystostigmine or diisopropylfluorophosphate to perfusing media. ACh, which is then not hydrolysed upon release can diffuse through the extracellular space to reach the tip of the push-pull cannula or the cup on the surface of the cortex.

The role of the extracellular space in the estimation of ACh released in vivo

As has been mentioned, diffusion of ACh through the extracellular space is the crucial point for determination of neurotransmitter release. If the push-pull cannula is placed in the brain ventricles (this technique is often used and will be described below) ACh additionally must diffuse through the ependyma and into the cerebrospinal fluid (CSF), where traces of neurotransmitter synthetized mainly non enzymatically have been found recently (3, 38). The extracellular space in the brain may be generally defined as a compartment between the membranes of neurons and glia cells. Measurement of its size may be done chemically (75) or by measuring electrical resistance (33) or using electron microscopy (33). For chemical estimation inulin is often used. This compound is not taken up by neurons and glia and thus remains in the extracellular space. The size of the extracellular space can be expressed in several different ways: ml/g dry weight or percent of wet weight — (22). It must be mentioned that there is still a large discrepancy in measurements of the size of the extracellular space from 0 to 40% of wet weight (cf. 22). Extracellular space estimated e.g. for the hippocampus using inulin has been determined as 8% of the wet weight and with electron microscopy only as 1.5% of the wet weight (49). Chemical compounds of low molecular weight can diffuse through the extracellular space, as the distance between the membranes of neurons and glia is some few hundred angstroms (75). It has also been reported that the extracellular space may change during anesthesia (76).

Influence of K⁺ and Ca⁺⁺ on ACh release

Stimulation dependent release of ACh by potassium ions has been found (6, 13, 41). Synaptic membranes are more permeable to K⁺ than Na⁺. Due to this property surface membrane potentials can be recorded, the size of which depends upon differences between intra- and extracellular concentration of potassium ions (8). Extracellular concentration of potassium ions rises following stimulation (47, 48) and may reach level of 12 mM. Further increment of the concentration
of potassium (e.g. spreading depression-47) may evoke increasing membrane depolarization followed by swelling (51). Potassium ions react in some way with membranes, changing their structure and facilitating the release of neurotransmitters which may be long lasting. Increase of extracellular potassium concentration is followed by calcium-dependent increase in postsynaptic miniature end plate potentials (MEPP) in the frog rectus abdominis (15, 68). The presence of potassium ions facilitates the interaction of Ca++ with the mechanisms underlying neurotransmitter release (30). These conclusions are further supported by data obtained from rat brain synaptosomes (1, 9, 36, 37). Increasing K+ ion concentration stimulates the uptake of calcium ions by synaptosomes. Simultaneously, increases in release of ACh and noradrenaline have been observed. Other mechanisms regulating ACh release have also been postulated (94). During nerve cell excitation, the increased concentration of Ca++ inside the cell temporarily inhibits the activity of the ion pump Na, K—ATPase, thus stimulating ACh release (94). However, it is not clear if ATPase (ATP phosphohydrolase) inhibition plays a direct or indirect role in this stimulation—dependent ACh release. Both sodium and potassium ions are located in nerve endings in a compartment sensitive to osmotic shock. On the other hand Ca++ ions are mainly bound by mitochondria in this compartment in an ATP-dependent manner (93). The various localization of these ions suggests different mechanisms for the regulation of their concentration in the extracellular space. So, the concentration of Na+ and K+ ions would be mainly regulated by osmotic strength and activity of the Na+-K+ pump. In the regulation of the concentration of Ca++ ions additionally mitochondria would participate. This is in agreement with the theory, according to which movement of synaptic vesicles towards the presynaptic membrane could be due to contraction of actin-like protein (59, 74). As for these processes calcium ions are crucial, their concentration inside the nerve cell may regulate ACh release. Influence of anoxia on the synthesis and release of ACh (55) and the flattening of the EEG (5, 16) as a result of disappearance of neuronal activity may be at least partially explained by the role of mitochondria in the ACh release process.

Thus it is clear that local changes in the concentration of K+ and Ca++ ions may regulate release of ACh and possibly of other neurotransmitter in different brain regions. The next question to be answered is: how fast this local increase in K+ concentration by nerve cell activity may spread out and influence surrounding areas of the brain. There are only few data on this problem. In the cat cerebral cortex potassium ions diffuse with a constant speed of 1.03 ± 0.16mm²/h (34).
As diffusion takes place through the extracellular space (11) its size and shape may have implication for neurotransmission (62).

**Axonal transport of ACh**

Axonal transport of ACh estimated by Haggendal and his co-workers (42) in the peripheral nervous system is about 5 mm/h which is too slow to maintain an adequate ACh level in nerve endings (80). On the other hand, as turnover of ACh in nerve endings of the CNS is very high and newly synthetized ACh is preferentially released (26), one can conclude that inhibitors of axonal transport would not significantly influence ACh release.

**The uptake of ACh**

As has been previously mentioned, inactivation of neurotransmitters takes place mainly by reuptake. Acetylcholine is mainly hydrolysed by AChE after being released (85). Reuptake of this neurotransmitter also exists and has to be taken into consideration in release experiments. Cerebral slices take up ACh from the medium, following AChE inhibition, in an energy-dependent way (73). Concentration of ACh in slices was six times higher compared to the surrounding medium. The uptake of both ACh and choline is inhibited by hemicholinium (27, 73, 82). This point is important as hemicholinium is often used in experiments in vitro as a drug specifically inhibiting choline uptake only.

ACh uptake varies according to the cell type, brain region (73), and concentration of this neurotransmitter in the extracellular space (66, 82). The latter is especially important as AChE is inhibited during push–pull cannula and cup estimation of ACh. Finally, high concentration of K+ ions in vitro inhibits ACh uptake (38). This again emphasizes the importance of potassium ions in the regulation of the ACh concentration in the extracellular space.

**The influence of choline concentration on ACh release**

Choline is one of the substrates for biosynthesis of ACh. It can not be synthetized in the brain and is supplied mainly by the liver via the blood stream (1). Injection of choline or its precursors (20, 43, 44, 70), or addition of these compounds to the diet (21) increase the amount of ACh synthetized in the rat brain. The observed increase depends on the particular brain region which suggests a correlation between the brain region and the efficiency of the choline uptake system (15). The nerve endings of the central nervous system possess two uptake systems for choline: with high and low affinity (60, 89). The high affini-
ty system is able to take up choline even if it is present in the extracellular space or synaptic cleft in very low concentration. On the other hand, the low affinity system can work only in a high choline concentration. It is very probable, that the low affinity system exists in all types of nerve endings. On the other hand, the high affinity, sodium-dependent system is supposed to act only in nerve endings from which ACh is released (96). It is especially interesting that a correlation between the amount of released ACh and choline uptake has been found (16, 83, 84).

More details about the regulation of ACh biosynthesis can be found in an earlier review (89).

Recent evidence (14) indicates that the concentration of K⁺ ions influences the tonus of smooth muscle in blood vessels in the brain. This might suggest a correlation between blood flow (regulated by K⁺) and the amount of choline delivered to the cells.

THE RULES AND CONDITIONS OF WORKING OF PUSH-PULL CANNULA

The push-pull cannula was first used in 1958 (35), but it was applied for the first time to the brain by Gaddum in 1961, (39). Figure 3 illustrates the push-pull cannula and perfusing system. Two thin tubes, either side by side, or one inside the other are introduced into the brain (67). The latter arrangement is used more often due to smaller brain damage. Recently (65, 81) a guide tube chronically implanted into the brain has been described and the push-pull cannula is introduced through this tube before each experiment. Outlets of both push-pull cannula tubes are separated outside of skull. Artificial cerebro-spinal fluid is pumped in with constant speed through the internal tube and pumped out through the external tube. A constant speed of perfusion is very important. Any decrease in the outflow produces an increase in the pressure in the brain tissue and may cause damage of the nerve cells surrounding the tip of the push-pull cannula. ACh can be measured in the outflow and consequently its release by brain tissue estimated per unit of time.

Little has been published about the optimal working conditions of the push-pull cannula. In 1975 Yaksh (95) described experiments in which the rat hypothalamus was injected with radioactive urea. Urea does not excite nerve cells, is not taken up or released by them, and mainly diffuses through the extracellular space. Following implantation of a push-pull cannula, urea was washed out with artificial cerebrospinal fluid. The largest outflow of radioactivity was found when the speed of perfusion was 80 μl/min. Further increase in the speed
of perfusion evoked a drop in the amount of eluted urea. The best results may be obtained (86), when the internal tube protrudes from the external one for a distance of 0.75 mm (see parameter “h” on Fig. 3). Perfusion can not be interrupted and even a one minute break evokes a long-lasting increase in the amount of the urea in the eluate.

Fig. 3. Schematic illustration of the push-pull cannula arrangement (according to 77, modified, not to scale); A, push-pull cannula implanted into brain tissue; 1, inside tube; 2, outside tube; 3, dental cement fixing the push-pull cannula with the screw to the bones of the skull; 4, skull; 5, brain tissue; B, the coupled syringe system; C, tip of the push-pull cannula (not to scale); h, the distance by which the inside tube protrudes from the outside one.

Damage of the brain tissue produced by implantation of the push-pull cannula and perfusion may both be important problems. As the push-pull cannula destroys some cells during implantation, its diameter should be as small as possible (about 1 mm). The damage due to perfusion is least if artificial cerebrospinal fluid is isotonic with natural CSF. Both hypo- and hypertonic solutions evoked large changes in brain tissue (95). Tubes used for perfusion should not be made from polyvinyl chloride (PVC), as this material contains some substances toxic to brain tissue (10). The contact area between the perfusion fluid and brain tissue was estimated after examination with an isotonic dye solution to be 1.3–1.5 mm³ (6, 23, 64, 92). In these conditions, degenerating cells were some 2 mm from the tip of the push-pull cannula (65).
The time from the implantation of the push-pull cannula to the beginning of the experiments also seems to be important. Introduction of the push-pull cannula (1.3 mm diameter) into the lateral ventricles of the rabbit brain evoked increase in CSF pressure 1 h after surgery (32). Five to six hours later, the pressure was about five times higher, as compared to normal conditions. It decreased 45 h later but even after 75 h the pressure was still twice as high as in controls. Variations in CSF pressure can be followed by other alterations in the tissue surrounding the push-pull cannula e.g. swelling of the brain, damage of the blood vessels and the changes evoked by local hypoxia (32). Fortunately, damage to the blood brain barrier appeared to be only partial with rapid recovery (32). A push-pull cannula system may be used to measure the amount of released neurotransmitters, as well as for introducing drugs to the brain (79).

Examples of the application of the push-pull cannula are described in part VI of this review.

VENTRICULAR PERFUSION

Ventricular perfusion is based on the principles of the push-pull cannula technique. Two tubes (not connected with each other) are introduced in two different places in the brain ventricles. Artificial cerebro-spinal fluid is pumped in by one of these tubes and pumped out by the other. The neurotransmitter released from nerve cells following stimulation spreads out through the extracellular space and reaches the brain ventricles from which it may be washed out during perfusion. Figure 4 shows two arrangements of ventricular perfusion.

As the ventricle walls are composed of different brain regions it is not possible to establish exactly from which brain structure release takes place. This disadvantage may be partially overcome by the addition of a dye to the perfusing fluid (66), which marks the perfusion area. As it has been mentioned before, released neurotransmitter has to diffuse to the ventricles through the ependyma. Changes in permeability evoked by experimental conditions may influence the composition of cerebrospinal fluid. It is clear from this short description that results obtained with this method are not precisely defined. Perfusion of ventricles has been used to investigate, among others: (i) ACh disappearance from brain ventricles (57), (ii) metabolism and transport of serotonin (69) and noradrenaline (4), (iii) changes in the concentration of electrolytes (12, 28) and in cAMP (54) in the cerebrospinal fluid. More details concerning this method can be found in the review by Myers (66).
Fig. 4. Scheme illustrating the measurement of the amount of neurotransmitter in brain ventricles (according to 67 modified); A, side view; B, front view; 1, inflow tube; 2, bones of the skull; 3, outflow tube; 4, lateral brain ventricle; 5, III brain ventricle; 6, IV brain ventricle. Arrows indicate the direction of perfusion. Dotted parts of the ventricles illustrate perfused area.

DESIGN AND APPLICATION OF THE CUP TECHNIQUE; ESTIMATION OF ACH

The cup technique was first described by McIntosh and Oborin in 1953 (58). The idea of this method is to measure the amount of neurotransmitter released into a small cup placed on the surface of the cortex (see Fig. 5). The area of cup depends on the species of animal and ranges from 0.3 cm² in the rat (2) to about 3 cm² in the sheep (63). The cup is filled with artificial cerebro-spinal fluid. Acetylcholine released from the nerve cells diffuses after AChE inhibition in all directions through the extracellular space and reaches, among other places, the cup on the cortical surface.

The cup technique can be used for acute and chronic experiments, just like the push-pull cannula. In acute experiments both cranium and underlying dura should be removed because only rabbit’s dura is fully permeable for ACh (77). However, an intact dura is essential to maintain brain integrity in chronic experiments. The cup is slightly pressed against the brain to avoid leakage of fluid. It is useful to pack the rim of the cup and surface of the cortex with parafin or silica gel,
if fluid escapes (18). The level of fluid in the cup should be stable during the experiment, but sometimes it may rise. This usually means that cerebro-spinal fluid is filling the cup due to pressure inside the brain. To overcome this, a syringe may be inserted in the large brain ventricle (63). The fluid in the cup should be mixed constantly with oxygen (63) or carbon dioxide mixture (77) to avoid inhibition of ACh diffusion by high concentration of this neurotransmitter close to the cortex surface. It is not necessary to mix the fluid in the cup, if the fluid is changed frequently.

Fig. 5. Diagram of the cup technique: (not to scale): 1, inflow tube; 2, outflow tube; 3, the cup; 4, dental cement fixing cup with the bones of the skull (5); 6, dura; 7, brain tissue. For detail explanation see text.

It is very useful to estimate, simultaneously with ACh release, diffusion of a neutral substance e.g. urea (23). Lack of changes in urea release and changes in ACh release following nerve cell stimulation suggests that the latter is specific. On the other hand, interpretation of the data is difficult as changes in the amount of urea released after stimulation have also been reported (91).

It is important to know the volume of brain tissue from which ACh may diffuse. It has been found with radioactive choline (18) that using a cup of 1 cm² crosssection (during a 10 min collection period) most of the ACh diffusing from the surface is limited by the rim of the cup and to a depth of 2 mm. The amount of ACh which diffuses from the depth greater than 2 mm sharply decreases. Similar results have been
found by Lancaster (56) during estimation of ACh diffusion through cat cortex slices. Moreover, to obtain any diffusion of ACh in his experiments it was necessary to inhibit AChE by 99%.

EXAMPLES OF APPLICATION OF THE PUSH-PULL CANNULA AND THE CUP TECHNIQUE

_Push-pull cannula_

Although the push-pull cannula technique is not generally used in neurochemical studies, this technique has provided some interesting results. Collier and Mitchell (23, 25) have found an increase in the release of ACh from the visual cortex of the rabbit following light stimulation. Treatment of the rabbit with a small stress increases the release of noradrenaline and serotonin from the olfactory bulb (19). Dependence of the amount of serotonin release on different phases of sleep strongly suggests participation of this neurotransmitter in sleep regulation. This technique has also been used to describe which parts of the hypothalamus of the monkey (64) and cat (18) release the largest amount of ACh. Recently this method was introduced for estimation of glutamic acid release from hippocampal slices (90).

Myers experiments deserve special attention (67). Two push-pull cannulae were introduced to identical brain structures of different animals. The outlet of the first push-pull cannula placed in the brain of one animal was connected with the inlet of the push-pull cannula of the brain of the second animal. The first animal was trained to exhibit a particular behavior. Different substances, among them probably neurotransmitters released during these tests from the brain of the first animal were washed out by the push-pull cannula and transported to the brain of the second animal. Similar behavior was than observed in the second animal.

From the results obtained with the push-pull technique presented in Table I, it can be seen that variability of the data is very high. The amount of ACh released (ng/ml of perfusing fluid) ranges from 0.1 (64), to 200 (see 25). This variability can not be explained by differences between animals or brain structures. On the other hand, one can suggest that the period of time between surgery and the start of the experiments is of importance. Short time (hours) is related to higher ACh release, whereas longer periods of time (days) correlate with lower ACh release (compare 24 and the rest of Table I). The influence of anesthesia on ACh release is also seen.
The amount of acetylcholine released in different brain areas of various mammals estimated with push-pull cannula technique.\textsuperscript{a} From introducing of push-pull cannula to the start of the experiment; \textsuperscript{b} In artificial cerebro-spinal fluid used for perfusion; \textsuperscript{c} The data are recalculated.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Brain area</th>
<th>Speed of perfusion $\mu$L/min</th>
<th>Time after surgery</th>
<th>AChE inhibitor/ eserine concentration g/ml\textsuperscript{b}</th>
<th>State of the animal</th>
<th>ACh released ng/ml/mln\textsuperscript{c}</th>
<th>References</th>
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<td>3h</td>
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<td>Conscious</td>
<td>200</td>
<td>25</td>
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<tr>
<td></td>
<td></td>
<td>33</td>
<td>7h</td>
<td>$10^{-4}$</td>
<td>Nembutal anesthesia</td>
<td>70</td>
<td>25</td>
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<tr>
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<td>Hypothalamus</td>
<td>30–50</td>
<td>5–7 days</td>
<td>$10^{-4}$</td>
<td>Conscious</td>
<td>0.1–0.5</td>
<td>64</td>
</tr>
<tr>
<td>(Macaca</td>
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<td>7 days</td>
<td>0</td>
<td>Conscious</td>
<td>0.5</td>
<td>29</td>
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<tr>
<td>mulatta)</td>
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<td></td>
<td></td>
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<td></td>
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<tr>
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<td></td>
<td></td>
<td>100</td>
<td>3–6 days</td>
<td>0</td>
<td>Conscious</td>
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<td>40</td>
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**Cup technique**

With the cup technique (58) correlation between electrical activity of the brain and the amount of ACh released has been found. Stimulation of the lateral geniculate body (part of the visual pathway in the brain), evoked an increase in ACh released from the visual cortex. Similar results were obtained following light stimulation of the retina (23). Frequency dependent increase of ACh release from the surface of the cortex has been found during electrical stimulation of the reticular formation (87). Using a slightly modified cup, Beani and Bianchi were able to determine changes in ACh release from the rabbit cortex up to 2 wk following surgery (18). Later the cup gets displaced from the skull due to osteolytic processes. Beani and co-workers have found a correlation between changes in EEG evoked by pentobarbital and amphetamine and changes in animal behavior and ACh release. According to these authors the dura has no influence on ACh release.

Szerb and his co-workers (88) found, using a pretrigeminal preparation of the cat (97), that there is a strong negative correlation between
cortical ACh output and ACh content. It means that cholinergic nerve terminals in the cat cortex are unable to maintain a stable level of ACh during its increased release. Recently (17) an influence of phosphatidylserine injected intravenously on ACh output from the rat brain cortex has been reported.

Fig. 6. Release of ACh from the cortex of freely moving rabbit during different states of arousal estimated with the push-pull cannula (according to 25 modified); A, anesthetized animal (implantation of push-pull cannula); B, conscious animal (exploration of the cage, drinking, eating); C, quiet animal; rare movements; D, active animal, E, quiet animal, F, anesthetized with Nembutal (30 mg/kg i.v.); G, death after lethal dose of Nembutal.

An attempt to correlate the amount of ACh released from the cortex and the type of behavior of the animal is shown in Fig. 6. It is, however, only possible to suggest that higher activity and state of arousal of the animal is accompanied by an increase in ACh release. On the other hand, a correlation between ACh released by different parts of the rabbit cortex and different behavioral tests performed by these animals has been demonstrated (77, 78). Some results obtained with the cup technique are illustrated in Table II.

The measurements of ACh release were mainly taken from visual and temporal cortices. In most experiments the dura had been removed but it is not clear if it really affects ACh release, as no special studies were performed. It is difficult to compare results obtained in various laboratories mainly due to the different experimental conditions. The period of time between the surgery and the start of the
The amount of acetylcholine released on the surface of some areas of the brain cortex of various mammals estimated with cup technique. *a* the data are recalculated; Eserine (about $10^{-4}$g/ml) in the cup was used in all experiments except (50, 88).

<table>
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<th>Time after surgery</th>
<th>State of the animal</th>
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<td>50</td>
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<td>Conscious</td>
<td>-</td>
<td>0.2-2.4</td>
</tr>
<tr>
<td></td>
<td>Auditory</td>
<td>0.5h</td>
<td>Allobarb. + urethane</td>
<td>0.3-1.3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Not specified</td>
<td>0.5h</td>
<td>Allobarb. + urethane</td>
<td>-</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30h</td>
<td>Allobarb. + urethane</td>
<td>-</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Concoius</td>
<td>1.6</td>
<td>Conscious</td>
<td>1.2</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1h</td>
<td>Conscious</td>
<td>1.7</td>
<td>2</td>
</tr>
<tr>
<td>Rat</td>
<td>Temporal</td>
<td>1h</td>
<td>Conscious</td>
<td>1.4</td>
<td>-</td>
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<tr>
<td>Sheep</td>
<td>Somatosensory</td>
<td>No information</td>
<td>Ether</td>
<td>1.3</td>
<td>-</td>
</tr>
<tr>
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<td>No information</td>
<td>Ether</td>
<td>1.3</td>
<td>-</td>
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experiment was usually a few hours but some authors often neglected to define it. It may be suggested that chronic experiments are more reliable than acute ones for estimation of ACh release. The different anesthetics and AChE inhibitors which have been used make it impossible to use any of these results as a reference and they may only be of use as relative data.

CONCLUDING REMARKS

The techniques presented for estimating ACh release in vivo have both benefits and drawbacks. Each must be used with proper perspective in accordance with the type of information they can yield. The method of ventricular perfusion is rather an introductory technique. If changes in the level of a compound appear following brain stimulation it may be suggested that this compound plays a role in the CNS. The push-pull cannula technique allows precise localization of the part of the brain responsible for the observed changes. Both techniques may be used not only for perfusion, but also for introducing drugs into brain tissue.

On the other hand, the cup technique is used for release experiments on the surface of the brain. Compared to the push-pull cannula, this technique is less convenient as: (i) it is not easy to use in chronic experiments, (ii) the temperature of exposed cortex in acute experiments should be maintained at the same level during the experiment which is not easy, (iii) introducing a push-pull cannula into the brain probably involves less stress than cup implantation.

If one compares Tables I and II it is obvious that the cup technique has been used more often. This technique is seemingly easier to perform. It may be concluded, that the potential possibilities of the push-pull cannula, for which the theoretical basis is now known, are still waiting to be used.

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REFERENCES


64. MYERS, R. D. and BELESLIN, D. B. 1970. The spontaneous release of 5-hydroxytryptamine and acetylcholine within the diencephalon of the unanesthetized Rhesus monkey. Exp. Brain Res. 11: 539–552.
push-pull method for the measurement of acetylcholine release in the rat brain.


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