Differences in the action of various drugs on striatal acetylcholine and choline content in rats killed by decapitation or microwave radiation

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The measurement of the acetylcholine content in various structures of rat brain yields information on the action of various drugs on the neuronal stores of the amine (Phillis, 1968; Szerb, Malik & Hunter, 1970; Guyenet, Agid & others, 1975). Some investigators maintain that changes in acetylcholine content reveal indirect information on its turnover rate (Stadler, Lloyd & others, 1973; Guyenet & others, 1975). Thus, the increase in striatal acetylcholine content elicited by agonists of dopamine receptors (Sethy & Van Woert, 1974; Ladinsky, Consolo & Garattini, 1974; McGeer, Grewaal & McGeer, 1974; Consolo, Ladinsky & Garattini, 1974; Guyenet, Agid & others, 1974; Rommelspacher & Kuhar, 1975) has been interpreted to indicate a decrease in its striatal turnover rate which is, perhaps, due to a decrease in the activity of striatal acetylcholine neurons. Injection of dopamine receptor antagonists (Guyenet & others, 1974; McGeer & others, 1974; Sethy & Van Woert, 1974; Rommelspacher & Kuhar, 1975) and cerebral hemisection (Rommelspacher & Kuhar, 1975) decrease striatal acetylcholine content. Perhaps, this decrease reflects an increase in its striatal turnover rate and an acceleration in the activity of cholinergic neurons (Guyenet & others, 1975). However, a decrease of striatal acetylcholine content does not always indicate an increase in its turnover rate. Antiacetylcholine drugs decrease the steady state concentration of brain acetylcholine without changing its turnover rate (Racagni, Cheney & others, 1975).

When the brain enzymes involved in acetylcholine metabolism are not inactivated instantaneously and irreversibly before assaying the amine content in brain, this parameter may change due to its rapid postmortem metabolism. The rate of this inactivation may vary according to the metabolic rates that were operative immediately before death.

When rats are killed with microwave radiation focussed to the head, the enzymes involved in the synthesis and degradation of acetylcholine are promptly inactivated (Guidotti, Cheney & others, 1974). We now show that when the rats are killed with microwave radiation the striatal acetylcholine content of rats injected with dopamine receptor agonists or antagonists remains unchanged. Thus the data presented in other reports (Sethy & Van Woert, 1974; Ladinsky & others, 1974; McGeer & others, 1974; Consolo & others, 1974; Guyenet, & others 1974; Rommelspacher & Kuhar, 1975) that striatal acetylcholine content increases in rats injected with dopamine receptor agonists or decreases in rats injected with haloperidol may be an artifact due to the procedure used to kill the rats. In this laboratory it has been shown that chlorpromazine, haloperidol, (+)-amphetamine, apomorphine, or L-dopa failed to change striatal acetylcholine content in rats killed by microwave radiation. In addition, it was found that chlorpromazine and haloperidol increased while (+)-amphetamine, apomorphine and L-dopa decreased the turnover rate of striatal acetylcholine (Trabucchi, Cheney & others, 1974, 1975).

Male Sprague Dawley rats (130–150 g) (Zivic Miller, Allison Park, Pa.) were killed following intraperitoneal injection of drugs either by focusing a beam of microwave radiation (2·0 KW; 2·45 GHz; 75 W cm^{-2}) for 2·4 s directly on the skull (Guidotti & others, 1974) or by decapitation. After decapitation, the brains were rapidly

removed and promptly placed on ice; the striata were dissected and homogenized without delay. Maximal time between decapitation and homogenization was 2 min. Acetylcholine and choline contents of striatum were determined according to Jenden & Hanin (1973).

Four drugs were injected intraperitoneally: a cholinergic receptor blocker, trihexylphenidyl (14 μmol kg^{-1}; 30 min); a dopaminergic receptor blocker, haloperidol (10 μmol kg^{-1}; 40 min); a cholinergic receptor stimulant, oxotremorine (9 μmol kg^{-1}; 30 min); and a dopaminergic receptor stimulant, apomorphine (10 μmol kg^{-1}, 20 min). The acetylcholine content in striatum was found to be 70–80% higher and choline content 70–80% lower in rats killed by microwave than in rats killed by decapitation (Tables 1, 2). This is in agreement with other reports (Schmidt, Speth & others, 1972; Guidotti & others, 1974; Stavinoha & Weintraub, 1974).

Trihexylphenidyl (Table 1) caused a 30% decrease in striatal acetylcholine content whether the rats were killed by decapitation or by microwave. However, haloperidol decreases the striatal content by 34% if the rats were decapitated but there was no change when the rats were killed by microwave (Table 1).

The striatal acetylcholine content of rats injected with oxotremorine increased by 88% if the rats were decapitated but this increase was smaller (23%) if the rats were killed by microwave (Table 2). Apomorphine increased the striatal content of the amine by 55% if the rats were decapitated but this increase failed to occur when the rats were killed by microwave. None of the drugs changed the striatal content of choline whether the rats were decapitated or killed by microwave radiation; however, after decapitation, the acetylcholine content was three times larger than that of rats killed by microwave. These results suggest that the striatal acetylcholine content in rats receiving dopamine receptor agonists and antagonists can change only if the rats are decapitated.

Since the enzymes that metabolize acetylcholine continue to function for a finite time in striatum of decapitated rats, the changes in its striata turnover rate elicited by drugs will be reflected as a difference in the content of the amine in the striatum. For instance, since haloperidol increases the turnover rate of striatal acetylcholine (Trabucchi & others, 1974) it appears probable that the decrease in content of the amine in the striatum that we found in decapitated rats (Table 1) may be due to its accelerated postmortem degradation (Guyenet & others, 1974; McGeer & others, 1974; Sethy & Van Woert, 1974). Apomorphine decreases this turnover rate (Trabucchi & others, 1975) and this may be the cause for the increase in the acetylcholine content of the striatum in rats killed by decapitation after treatment with

Table 1. *Acetylcholine and choline content in striatum of rats killed by decapitation or by microwave radiation following administration of trihexylphenidyl (14 μmol kg^{-1}, i.p.; 30 min) and haloperidol (10 μmol kg^{-1}, i.p., 40 min)*

	Microwave		Decapitation	
	Acetylcholine (nmol mg^{-1} protein)	Choline	Acetylcholine (nmol mg^{-1} protein)	Choline
Control	0·57 ± 0·040	0·32 ± 0·021	0·34 ± 0·023	0·98 ± 0·14
Trihexylphenidyl	0·40 ± 0·043*	0·31 ± 0·019	0·24 ± 0·037*	0·95 ± 0·13
Haloperidol	0·50 ± 0·049	0·27 ± 0·015	0·20 ± 0·047*	1·0 ± 0·10

Values represent mean ± s.e.m. of at least 5 determinations.
* $P < 0.05$.

Table 2. *Acetylcholine and choline contents in striatum of rats killed by decapitation or by microwave radiation following administration of oxotremorine (9 μmol kg⁻¹, i.p.; 30 min) and apomorphine (10 μmol, i.p.; 20 min).*

	Microwave		Decapitation	
	Acetylcholine	Choline	Acetylcholine	Choline
	(nmol mg⁻¹ protein)		(nmol mg⁻¹ protein)	
Control	0·60 ± 0·050	0·33 ± 0·029	0·33 ± 0·030	1·06 ± 0·11
Oxotremorine	0·74 ± 0·066*	0·33 ± 0·053	0·62 ± 0·084*	1·01 ± 0·087
Apomorphine	0·66 ± 0·041	0·28 ± 0·048	0·51 ± 0·043*	1·13 ± 0·072

Values represent mean ± s.e.m. of at least 5 determinations.
* $P < 0.05$.

apomorphine (Table 2) (Sethy & Van Woert, 1974; Ladinsky & others, 1974; McGeer & others, 1974; Consolo & others, 1974; Guyenet, & others, 1974). Since after microwave radiation the striatal acetylcholine content in rats given haloperidol (Table 1) or apomorphine (Table 2) was not changed, we infer that the striatal acetylcholine content *in vivo* is not changed by either antagonists or agonists of dopamine receptors. A decrease in the content cannot be taken as an index of an increase in striatal turnover because trihexylphenidyl decreases its striatal content without changing its striatal turnover rate (Racagni & others, 1975).

In conclusion, changes in the turnover rate of striatal acetylcholine elicited by drugs cannot be predicted by a decrease or an increase in striatal acetylcholine content measured in rats killed by decapitation.

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