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SOME NEUROCHEMICAL ASPECTS OF THE DEPRESSANT ACTION OF γ-BUTYROLACTONE ON THE CENTRAL NERVOUS SYSTEM

BY

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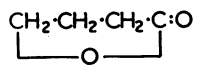
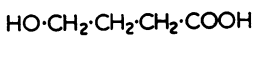
γ-Butyrolactone, a depressant drug of the central nervous system, has been investigated for its ability to alter brain levels of 5-hydroxytryptamine, γ-aminobutyric acid and acetylcholine in mice and rats; of these three compounds, only acetylcholine was changed in amount. Levels of acetylcholine in the cerebral cortex were increased by γ-butyrolactone with a time-course which closely followed the depressed state of the animal. Indirect evidence has been presented to show that in the mid-brain and brain stem the change in acetylcholine level induced by γ-butyrolactone is sharply localized in an area of the mesencephalon that contains the corpora quadrigemina.

Rubin & Giarman (1947), in an investigation of the chemotherapeutic effect of various lactones in influenza virus infection, observed that mice given γ-butyrolactone behaved as if anaesthetized, and this condition was later shown to be characterized in the cat by complete abolition of electrical activity in the neocortex (Giarman, 1948). Benda & Perles (1960) have presented results of experiments with rats, pigeons and rabbits, which confirm this depressant action of γ-butyrolactone on the central nervous system. Similar depressant effects in animals and in man, described as anaesthesia, have been reported for the product of alkaline hydrolysis of γ-butyrolactone, γ-hydroxybutyrate (Laborit, Jouany, Gérard & Fabiani, 1960; Laborit, Kind & Régil, 1961; Blumenfeld, Suntag & Harmel, 1962). Jenney, Murphree, Goldstein & Pfeiffer (1962) have demonstrated recently with human volunteers that both γ-butyrolactone and γ-hydroxybutyric acid produce sleep and light anaesthesia, followed, on awakening, by dissociation of the alert electroencephalographic pattern and behaviour.

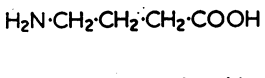
In view of the changes in brain acetylcholine level (Richter & Crossland, 1949; Giarman & Pepeu, 1962) and the changes in cerebral 5-hydroxytryptamine level (Anderson & Bonnycastle, 1960; Schanberg & Giarman, 1962) that result from the administration of certain central nervous depressant drugs, it was of interest to investigate the effect of γ-butyrolactone on the brain levels of these two compounds. In addition, the similarity in structure of γ-butyrolactone, γ-hydroxybutyric acid and



γ-Butyrolactone



γ-Hydroxybutyric acid



γ-Aminobutyric acid

the brain constituent, γ-aminobutyric acid prompted an extension of this investigation to include studies on the influence of γ-butyrolactone on the levels of γ-aminobutyric acid in the brain.

METHODS

Adult albino mice (Swiss, 15 to 30 g) and rats (Blue Spruce and Charles River, 150 to 250 g) were used. Experimental and control animals were of the same weight and strain. All were given 725 mg of γ-butyrolactone (Mathieson, Coleman & Bell) per kg of body weight by the intraperitoneal route; the drug had a boiling point of 91 to 93° C at 17 mm Hg. In most experiments, animals were killed by decapitation 15 to 30 min after the drug had been administered, at a time when the central nervous depression had developed fully.

Determination of acetylcholine. The acetylcholine was extracted by a modification of the method of Smallman & Fisher (1958); determinations were done on pooled samples of three mouse brains each. Each rat brain, after quick removal from the cranium, was divided into three parts: cortex (including some sub-cortical material), rostral mesencephalon, and posterior mesencephalon and telencephalon; the cerebellum, pituitary gland and olfactory bulbs were discarded. In six animals the line of partition of the mesencephalon and the brain stem was made rostral to the superior colliculi, while in another six this partition was made caudal to the inferior colliculi. Each of these subdivisions of brains from experimental and control rats was treated, as described above, for extraction of acetylcholine.

Bioassay of acetylcholine. Two preparations were used for the estimation of acetylcholine.

(1) Extracts of mouse brain were assayed on a section of the terminal ileum of the guinea-pig, suspended in Tyrode solution (containing morphine, 5 mg/l., and neostigmine, 1 μg/l.), bubbled with a mixture of 97% O_2 and 3% CO_2, and maintained at 30° C. Semi-isometric recordings were made on a Gilson mini-polygraph by means of a force-displacement transducer. The assay was conducted as a three-point assay with two knowns. The time-cycle was 30 sec exposure and 90 sec rest.

(2) Extracts of the sub-divisions of rat brains were assayed on frog isolated rectus abdominis muscle preparations, suspended in frog-Ringer solution (containing physostigmine, 10 μg/l.), bubbled with air and kept at room temperature. The recording system was as described above, except that a stretching device brought the muscle to its original base-line length. The content of acetylcholine was estimated by twice bracketing the unknown between various known amounts of standard acetylcholine chloride. The time cycle was 90 sec exposure and 3.5 min rest. All values of acetylcholine are expressed in terms of the chloride.

Determination of 5-hydroxytryptamine. Pooled rat brains (two to each sample) were homogenized in isotonic sucrose solution and particulate and supernatant fractions were obtained by high-speed centrifugation according to the method of Schanberg & Giarman (1962). Each fraction was extracted for 5-hydroxytryptamine with alkaline butanol, according to the method of Bogdanski, Pletscher, Brodie & Udenfriend (1956), and spectrophotofluorimetric measurements (395 mμ activation; 550 mμ emission) were made. All values of 5-hydroxytryptamine are expressed in terms of the base.

Determination of γ-aminobutyric acid. Pooled mouse brains (three to a sample) were used. A modification of the ethanolic extraction of γ-aminobutyric acid reported by Wallach (1960) was followed; the content of the drug in the extracts was estimated by an assay which uses transaminase prepared from *E. coli* (Wallach, 1960).

RESULTS

Acetylcholine

The rise in total cerebral acetylcholine in the mouse produced by doses of γ-butyrolactone which depress the central nervous system (Table 1) is about the same size as that reported for the rat (Giarman & Pepeu, 1962) and found by us for depression of the mouse and rat induced by pentobarbitone.

TABLE 1

CHANGE IN TOTAL CEREBRAL ACETYLCHOLINE PRODUCED BY γ-BUTYROLACTONE IN THE MOUSE

Acetylcholine contents are means with standard deviations

Chemical agent	Dose (mg/kg)	Time of killing (min)	Number of determinations	Content of acetylcholine (μg)	Change (%)	P
None	—	—	5	1·35±0·21	—	—
γ-Butyrolactone	750	15	5	1·95±0·26	+44	<0·05

A generalization that appears to be validated by a number of reports is that the elevation in brain acetylcholine associated with central nervous depressant drugs is correlated directly with the degree of depression; accordingly, the time-course of the rise in acetylcholine induced by γ-butyrolactone appeared to be of some significance. As shown by Fig. 1, alteration in level of acetylcholine follows closely the development of the depressed state of the animal: the peak of the rise in acetylcholine occurs at 15 min when central nervous depression is deepest, and this is

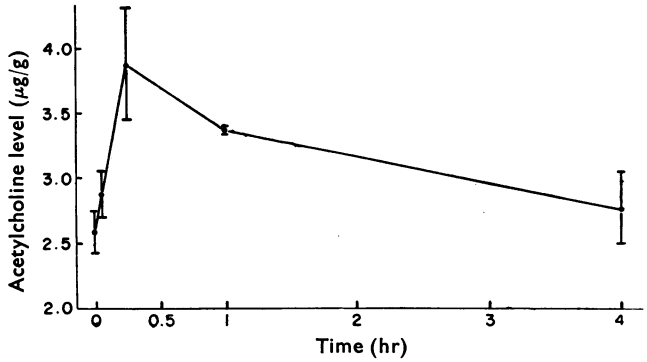


Fig. 1. Changes in acetylcholine level in mouse brain after administration of γ-butyrolactone (750 mg/kg). Vertical bars indicate standard deviations.

followed by a gradual decline to the time when the animals regain the righting reflex (after 3 hr).

During the course of experiments designed to uncover the pattern of change in acetylcholine level in different regions of the brain after administration of γ-butyrolactone, it became apparent that the degree of change observed in the rostral and posterior portions of the mid-brain and the brain-stem was determined by the presence or absence of the corpora quadrigemina and the structures underlying that area. A number of experiments were done, therefore, to establish this point (Table 2). It is clear that that portion of the mid-brain and brain-stem which contained the corpora quadrigemina demonstrated the elevation in acetylcholine induced by γ-butyrolactone while the other portion showed no significant change.

5-Hydroxytryptamine and γ-aminobutyric acid

Tables 3 and 4 demonstrate that γ-butyrolactone has no influence on the levels of 5-hydroxytryptamine or of γ-aminobutyric acid in the brain, or on the subcellular

TABLE 2

REGIONAL DISTRIBUTION OF CHANGES IN ACETYLCHOLINE LEVEL IN THE BRAIN-STEM OF THE RAT AFTER TREATMENT WITH γ-BUTYROLACTONE

" Brain-stem " refers to that part of the brain remaining after removal of the cerebellum and cerebral cortex. Levels of acetylcholine are means with standard errors. GBL=γ-butyrolactone. The values of *P* for significance of mean differences were calculated by the Student *t*-test

Area of brain	Number of expts.	Levels of acetylcholine (μg/g) in Untreated	GBL-treated	Change (%)	*P*
Cortex	12	1·64±0·13	3·23±0·19	+96	0·05
Combined " brain stem "	12	2·49±0·19	3·14±0·19	+26	0·05
Upper " brain stem " (without corpora quadrigemina)	6	2·76±0·34	2·67±0·33	−3	0·10
Upper " brain stem " (with corpora quadrigemina)	6	2·44±0·34	3·47±0·23	+42	0·05
Lower " brain stem " (without corpora quadrigemina)	6	2·68±0·27	2·61±0·27	−3	0·10
Lower " brain stem " (with corpora quadrigemina)	6	2·23±0·25	3·21±0·34	+44	0·05

TABLE 3

INFLUENCE OF γ-BUTYROLACTONE ON SUBCELLULAR DISTRIBUTION OF 5-HYDROXYTRYPTAMINE IN THE RAT BRAIN

Subcellular fractions were obtained by subjecting homogenates in isotonic sucrose to 100,000 *g* for 20 min. Values of 5-hydroxytryptamine are means of whole-brain contents

Treatment	Number of deter-minations	5-Hydroxytryptamine (mμg) in Supernatant fraction	Particulate fraction	Total	% of total in supernatant fraction
Controls	3	155	523	678	22·8
γ-Butyrolactone (750 mg/kg, 15 min)	3	153	545	698	22·4

TABLE 4

CHANGES IN LEVEL OF γ-AMINOBUTYRIC ACID IN MOUSE BRAIN AFTER γ-BUTYROLACTONE AND AMINO-OXYACETIC ACID

Levels of γ-aminobutyric acid are means with standard errors

Treatment	Number of determinations	Level of γ-aminobutyric acid (μmole/g)
Control	4	1·98±0·08
γ-Butyrolactone (750 mg/kg, 15 min)	3	1·91±0·16
Amino-oxyacetic acid (50 mg/kg, 4 hr)	2	8·50

distribution of 5-hydroxytryptamine. It was of interest to find that amino-oxyacetic acid, a compound shown by Wallach (1960) to inhibit γ-aminobutyric acid-α-ketoglutarate transaminase, caused a marked elevation in the brain level of γ-aminobutyric acid. This finding agrees with results with amino-oxyacetic acid reported by Wallach (1960).

DISCUSSION

The increase in acetylcholine levels in whole brain after the administration of γ-butyrolactone follows the same pattern as that described by Richter & Crossland (1949) for other central nervous depressants. γ-Butyrolactone, however, leads to differences in regional brain changes in acetylcholine level. The most marked change appears in the cerebral cortex, and is similar to changes seen with large doses of other anaesthetic agents (Richter & Crossland, 1949). Whether such differences should be correlated with differences in human clinical behaviour induced by γ-butyrolactone, in contrast to other anaesthetic agents, is as yet an unanswered question.

The changes in acetylcholine levels elicited by γ-butyrolactone in the "brain stem," that is the brain remaining after removal of the cerebellum and cerebral cortex, are of great interest. In this whole structure γ-butyrolactone induces elevations in acetylcholine level similar in size to those due to other anaesthetic drugs (Crossland & Merrick, 1954). If this structure is cut into two parts, however, only that portion which contains the corpora quadrigemina shows a significant rise in acetylcholine after γ-butyrolactone. This finding suggests that the region of the corpora quadrigemina contains those neuronal structures that respond most markedly to γ-butyrolactone by an altered metabolism of acetylcholine and, perhaps, to other central nervous depressant drugs. The core of the "brain stem" underlying the area of the corpora quadrigemina contains some of the neurones and their processes that constitute the reticular formation. It is well-documented that the reticular formation shows a marked change in electrophysiological activity upon the administration of many drugs which depress the central nervous system (Killam, 1962). It is tempting to link these sharply localized changes in acetylcholine level with functional changes known to be induced by drugs in this region. In this context, cholinergic mechanisms in the reticular formation have been indirectly implicated (Exley, Fleming & Espelien, 1958). Our results may provide more direct evidence for such mechanisms, if we assume that the change in acetylcholine level are localized in the core and not in the periphery of the quadrigeminal region. Investigations of this possibility should be undertaken.

The failure of γ-butyrolactone to alter either the levels or the subcellular distribution of cerebral 5-hydroxytryptamine is not unexpected. While administration of certain central nervous depressant drugs has been associated with changes in brain 5-hydroxytryptamine level (Anderson & Bonnycastle, 1960; Schanberg & Giarman, 1962), this response is not believed to be elicited by all central nervous depressant drugs (Schanberg & Giarman, 1962).

The failure of γ-butyrolactone to influence γ-aminobutyric acid levels in the brain might be considered somewhat more unexpected than its lack of effect on 5-hydroxytryptamine levels. Recently, Tsuji, Balagot & Sadove (1963) have reported a 34% reduction of the brain γ-aminobutyric acid level in rats anaesthetized with pentobarbitone, but the statistical significance of this result was not determined. This finding would appear to be anomalous, since an increased level of γ-aminobutyric acid, which generally depresses neuronal excitability (Purpura, Girado, Smith, Callan & Grundfest, 1959), might be expected in a brain depressed by barbiturates. In this

context, it is of interest that the great elevation of brain γ-aminobutyric acid level produced by amino-oxyacetic acid is accompanied by a generally depressed, but not anaesthetized, animal. There are clear differences between the depressed state produced by amino-oxyacetic acid and that produced by γ-butyrolactone, which fact agrees with our finding that γ-butyrolactone does not alter brain γ-aminobutyric acid levels.

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