Biochemical Pharmacology, Vol. 21, pp. 625–634. Pergamon Press, 1972. Printed in Great Britain

DRUG-INDUCED ALTERATIONS IN RESPIRATION OF RAT BRAIN CORTEX AND STRIATUM SLICES IN A CARBON DIOXIDE–BICARBONATE-BUFFERED MEDIUM*

GEORGE B. WEISS, LEIF HERTZ† and FRANK R. GOODMAN

Department of Pharmacology, University of Texas Southwestern Medical School, Dallas, Texas 75235, U.S.A.

(*Received* 23 *May* 1971; *accepted* 9 *July* 1971)

Abstract—Rates of oxygen utilization were measured with an oxygen electrode in rat brain cortex and striatum slices. Values obtained for respiratory rates in cortex (approximately 85 μmoles/hr/g wet weight) and striatum (about 43 μmoles/hr/g) were similar in both bicarbonate and tris-buffered solutions. Addition of potassium chloride (to 70 mM) elicited an increase in oxygen utilization in both preparations. Exposure to procaine (10 mM) reduced the respiration rate to levels substantially below control values and prevented the K^+-induced increase in respiration rate. Pentobarbital sodium (1 mM) and, perhaps to a lesser extent, caffeine (5 mM) also decreased the control respiration rates and inhibited the K^+-induced stimulation of respiration rates. Nicotine (0·67 mM) had no effect on control or K^+-stimulated respiration rates in cortex or striatum slices. Effects of procaine appeared to be additive with those of caffeine and pentobarbital, and are probably attained by different cellular mechanisms. Observation of similar respiratory rates in bicarbonate and tris solutions also suggests that correlations may be attempted between brain tissue respiration studies in CO_2-free media and measurements of ionic parameters in the presence of bicarbonate-buffered solutions.

IONIC concentrations and movements in brain tissue have often been determined following incubation in bicarbonate-buffered solutions, whereas methodological considerations have resulted in the performance of oxygen uptake measurements in the absence of CO_2 and bicarbonate. The importance of CO_2 for at least some aspects of brain, nerve and retinal metabolism has been demonstrated,[1–4] and replacement of the bicarbonate–CO_2 buffer in the medium with a Tris buffer leads to a reduction of the potassium content in brain slices from 70–55 μmoles/g final wet weight.‡ To correlate ionic parameters with oxygen uptake under similar conditions, it would be necessary to determine oxygen uptake in the presence of CO_2. Determinations of this type can be performed readily with an oxygen electrode either in the presence or the absence of CO_2.[5]

Considerable information is available about effects evoked by barbiturates and local anesthetics on oxygen uptake[6–10] and ion movements[11,12] in stimulated and unstimulated brain cortex slices. To examine effects of these agents on oxygen uptake under the conditions of the present study, a barbiturate (pentobarbital) and a local anesthetic (procaine) were reinvestigated at concentrations high enough to observe

* This study was aided by a grant from the American Medical Association Education and Research Foundation.

† Present address: Department of Biochemistry A, University of Copenhagen, 30 Juliane Mariesvej, 2100 Copenhagen, Denmark.

‡ H. Lund-Andersen and L. Hertz, unpublished experiments.

inhibition of K^+-induced stimulation of respiration and of resting respiration. In addition, the effects of caffeine and nicotine (two widely used agents with extensive central nervous system actions) on oxygen uptake were ascertained. Caffeine has a number of actions on Ca^{2+} movements in muscle systems[13–15] as well as an inhibitory effect on rat brain phosphodiesterase,[16] an enzyme which inactivates cyclic AMP. Nicotine also affects calcium movements[17–20] as well as intracellular pH[21,22] in muscle systems. However, little is known about the effects of caffeine and nicotine on ion movements and metabolism in brain tissues *in vitro*. Since the effects observed with different agents may vary from one brain area to another, slices from both the cortex and the corpus striatum were used routinely.

METHODS

Brain slices used in these experiments were obtained from male Wistar rats weighing between 180–250 g. Rats were lightly anesthetized with ether and decapitated. The brain was removed and placed on moistened filter paper placed on ice. One superficial cortical slice (0·5 mm thickness) was prepared from each hemisphere (using apparatus specifically designed for preparation of brain slices)[23] and both corpus striatum areas were removed and sliced in halves as previously described.[24] The striatum slices are identical to those termed "caudate" in the previous study.[24] The slices were weighed on a Federal-Pacific torsion balance and equilibrated in test tubes containing the incubation medium for 5–40 min. No significant differences in effects measured were noted for equilibration periods of up to 1 hr. The incubation medium (bicarbonate solution) contained 120 mM NaCl, 15 mM $NaHCO_3$, 5 mM KCl, 1·5 mM $CaCl_2$, 1·0 mM $MgSO_4$ and 6 mM glucose. In some experiments, the $NaHCO_3$ was replaced with 20 mM of tris(hydroxymethyl)aminomethane (Tris solution). All solutions were mixed with demineralized water, and incubated slices were aerated intensely[25] with either 95% O_2 and 5% CO_2 (bicarbonate solution) or 100% O_2 (Tris solution). The pH of the solutions was 7·3 and the temperature was 37°. Agents used were excess potassium chloride (to 70 mM), procaine hydrochloride (10 mM), pentobarbital sodium (1 mM), caffeine (5 mM), and nicotine (0·67 mM). All agents were added to solutions in appropriate amounts on the day that each experiment was performed, and pH was subsequently adjusted back to 7·3 when necessary.

Before decapitation, a 3-ml chamber monitored with a Clark oxygen electrode[5] was filled with incubation solution and thoroughly saturated with O_2 from the aeration source appropriate for the solution. The stability of the oxygen tension in the absence of respiring tissue was controlled immediately after preparation of the tissue, and either both of the cortical slices (one from each hemisphere) or all four of the striatum halves were inserted rapidly. The average weights (in milligrams $\pm$ S.E.M.) of the tissues inserted were 103 $\pm$ 4 for the cortex slices and 78 $\pm$ 3 for the striatum preparations. Electromagnetic stirring was employed,[5] and an outlet for excess medium in the stopper of the electrode chamber was sealed with parafilm during all measurements. After the decline in oxygen tension evoked by the respiring tissue in 3 ml of medium had been followed for 2–5 min, the chamber was opened and a small volume of stock solution of the agent to be studied was added. Additions of this type could be performed as often as 4 times during an experiment. Control additions of equivalent volumes of medium alone did not alter the rate of decline in oxygen tension.

The oxygen tension measurement records were obtained with a Sargent Model

SRG recorder (sensitivity, 10 mV). The deflection corresponding to saturation (i.e. 760 mm Hg with pure oxygen and 722 mM Hg with 95% O_2/5% CO_2) was recorded in most of the individual experiments, and that corresponding to the total absence of oxygen (0 mm Hg) was read twice daily after addition of sodium dithionite. The decline in oxygen tension during each experimental period was measured graphically and the oxygen consumption was calculated in the conventional manner.[5] The amount of oxygen consumed is expressed in terms of μmoles per gram initial wet weight per hour.

RESULTS

The parameter measured was the rate of decline in oxygen tension during the experiment. Prior to addition of stimulatory or inhibitory agents, it was necessary to determine whether changing levels of oxygen tension during an experiment had any influence on the tissue respiratory rate. In Fig. 1, typical records demonstrate the changes in oxygen tension observed with cortex and striatum slices in bicarbonate solution in the presence and absence of high potassium. The rate of respiration was increased in K^+-stimulated slices from both cortex and striatum. In the striatum

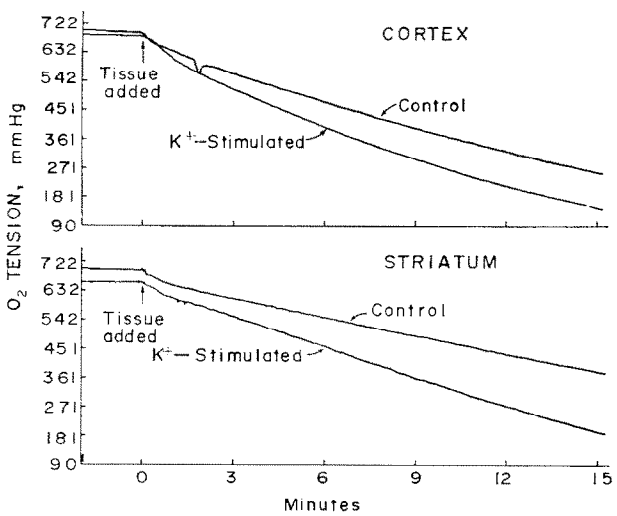

FIG. 1. Tracings of typical records obtained with the oxygen electrode and showing the rate of oxygen utilization in bicarbonate solution for both control and potassium-stimulated slices from rat brain cortex and striatum.

slices, the change in oxygen tension was rectilinear with time until tension fell to less than half of the saturated value, whereas some deflection was observed for cortex slices even during this earlier portion of the experiment. Thus, some reduction in cortical oxygen uptake occurs as oxygen tension is lowered, and the underestimate of respiratory activity which results is even greater in the K^+-stimulated cortical slices.

A major advantage of the oxygen electrode is the ability to measure O_2 uptake in the presence of CO_2. The comparison of measurements obtained in either bicarbonate–CO_2-buffered media or Tris-buffered solutions is included in Table 1. No statistically

TABLE 1. COMPARISON OF CORTEX AND STRIATUM SLICE RESPIRATION RATES IN BICARBONATE AND TRIS-BUFFERED SOLUTIONS UNDER CONTROL CONDITIONS AND AFTER ADDITION OF POTASSIUM AND OF PROCAINE*

Brain area slices	Solution	Control Respiration rate (μmoles/hr/g)	Potassium (70 mM) Respiration rate (μmoles/hr/g)	Potassium (70 mM) Increase from control (%)	Potassium (70 mM) + procaine (10 mM) Respiration rate (μmoles/hr/g)	Decrease from K^+-treated (%)	Decrease from control (%)
Cortex	Bicarbonate	85·4 ± 5·7	104·6 ± 5·0	22·5	50·9 ± 3·9	51·3	40·4
Cortex	Tris	85·1 ± 7·6	113·7 ± 7·2	33·6	57·0 ± 2·6	49·8	33·0
Striatum	Bicarbonate	44·5 ± 3·0	68·8 ± 6·2	54·6	35·8 ± 3·4	48·0	19·6
Striatum	Tris	42·4 ± 2·0	67·5 ± 3·3	59·2	31·8 ± 2·5	52·3	25·1

* Figures for respiration rates represent mean values ± S.E.M. for five slices (striatum, bicarbonate) or six slices (all others).

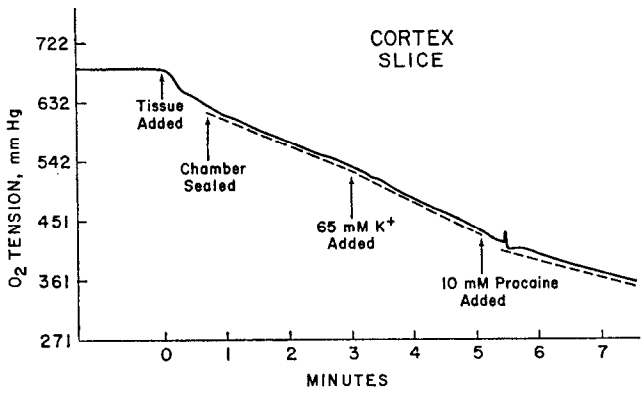

FIG. 2. Tracing of a typical recording which illustrates the alterations observed in the rate of decline in oxygen tension in slices from rat cortex when the K^+ concentration was increased to 70 mM and, subsequently, procaine (10 mM) was added to the bicarbonate-containing incubation solution.

significant differences were found for slices incubated in the two different solutions either before or after addition of 65 mM potassium. Also, the addition of 10 mM procaine depressed the stimulated respiration rate to the same degree in both cases. A typical recording of the changes observed in respiration rate in one of these experiments with cortex slices is illustrated in Fig. 2. Since no difference was observed, all subsequent experiments were performed with the bicarbonate–CO_2-buffered solution. The procaine-induced inhibition of tissue respiration was of greater magnitude than the preceding increase due to K^+-stimulation. To measure the inhibitory effects of 10 mM procaine directly, this agent was also added in the absence of increased K^+. Under these conditions, the rate of oxygen consumption was depressed to similar levels as were seen in the K^+-stimulated tissues, and subsequently raising the K^+ concentration to 70 mM did not alter the respiration rate (Table 2). Thus, the final respiration rate after addition of procaine and increased K^+ was not changed by reversing the sequence in which these two agents were added to the incubation media.

In order to obtain information about the actions of other agents on both unstimulated and stimulated respiration, a similar procedure was followed. Control rates were determined and then the desired agent was added. After allowing time for the

TABLE 2. EFFECT OF PROCAINE AND POTASSIUM ON CORTEX AND STRIATUM RESPIRATION RATES WHEN PROCAINE IS ADDED PRIOR TO POTASSIUM*

Brain area slices	Control Respiration rate (μmoles/hr/g)	Procaine (10 mM) Respiration rate (μmoles/hr/g)	Procaine (10 mM) Decrease from control (%)	Procaine (10 mM) + potassium (70 mM) Respiration rate (μmoles/hr/g)
Cortex	84·4 ± 6·0	59·1 ± 3·9	30·0	58·7 ± 3·0
Striatum	51·5 ± 1·9	35·9 ± 1·7	30·4	36·4 ± 2·6

* Figures for respiration rates represent mean values ± S.E.M. for six slices.

TABLE 3. EFFECT OF PENTOBARBITAL ON CORTEX AND STRIATUM RESPIRATION RATES*

Brain area slices	Control Respiration rate (μmoles/hr/g)	Pentobarbital sodium (1 mM) Respiration rate (μmoles/hr/g)	Decrease from control (%)	Pentobarbital sodium (1 mM) + potassium (70 mM) Respiration rate (μmoles/hr/g)	Increase from pentobarbital (%)	Decrease from control (%)
Cortex	90·4 ± 2·0	70·5 ± 7·2	22·0	81·1 ± 4·2†	15·1	10·2
Striatum	54·9 ± 3·0	42·3 ± 2·3	23·0	49·6 ± 1·6	17·2	9·7

* Figures for respiration rates represent mean values ± S.E.M. for five slices.
† Not significant ($P > 0.05$); all other comparisons are significantly different ($P \leq 0.005$) when treated as paired values.

TABLE 4. EFFECT OF CAFFEINE ON CORTEX AND STRIATUM RESPIRATION RATES*

Brain area slices	Control Respiration rate (μmoles/hr/g)	Caffeine (5 mM) Respiration rate (μmoles/hr/g)	Decrease from control (%)	Caffeine (5 mM) + potassium (70 mM) Respiration rate (μmoles/hr/g)	Increase from caffeine (%)	Increase from control (%)
Cortex	88·6 ± 7·6	81·0 ± 8·3	8·6	97·2 ± 7·3	20·1	9·8
Striatum	53·5 ± 2·4	45·4 ± 2·3	15·0	63·5 ± 4·3	39·9	18·9

* Figures for respiration rates represent mean values ± S.E.M. for six slices. All comparisons are significant ($P < 0.025$ or better) when treated as paired values.

establishment of a new rate, K^+ was added. The effects of pentobarbital sodium, caffeine and nicotine were measured in cortex and striatum slices in this manner. Pentobarbital (1 mM) significantly decreased unstimulated respiration by about the same magnitude in cortex and striatum slices (Table 3). Subsequent addition of K^+ (to 70 mM) caused a distinct but relatively small increase in average oxygen uptake rates which rose to values slightly lower than unstimulated control values. Caffeine (5 mM) had possible slight depressant effects on both stimulated and unstimulated respiration (Table 4). In both cortex and striatum slices, the stimulated respiration rate was higher than the control values but not as high as values for stimulated respiration rates in the absence of caffeine (Table 1). Nicotine (0·67 mM) had no effect on unstimulated respiration rates and did not reduce the stimulatory effects of added K^+ (Table 5), which were of the same magnitude as in the absence of nicotine (Table 1).

TABLE 5. EFFECT OF NICOTINE ON CORTEX AND STRIATUM RESPIRATION RATES*

Brain area slices	Control Respiration rate (μmoles/hr/g)	Nicotine (0·67 mM) Respiration rate (μmoles/hr/g)	Nicotine (0·67 mM) + potassium (70 mM) Respiration rate (μmoles/hr/g)	Increase from control (%)
Cortex	99·1 ± 5·7	99·1 ± 5·7	123·5 ± 9·9	24·6
Striatum	57·6 ± 1·5	57·4 ± 2·2	88·0 ± 4·3	52·8

* Figures for respiration rates represent mean values ± S.E.M. for three slices.

In many of the experiments performed with pentobarbital, caffeine and nicotine, procaine (10 mM) was also added after the effects of the initial agent and of 70 mM K^+ had been ascertained. Since potassium had no effect in the presence of procaine (Table 2), the values obtained for procaine (and K^+) are compared with those obtained with the other agents prior to exposure to both added potassium and procaine (Table 6). The first column shows the initial control values obtained, the second gives the rate of respiration after addition of the drug in question (or no drug), and the third indicates the rate of oxygen uptake after further addition of procaine (and K^+). The differences between the procaine-inhibited respiratory rates and the rates of oxygen uptake preceding addition of procaine (and K^+) are given in the fourth column, and those between the respiratory rates inhibited by procaine and the control values are listed in the final column. The addition of procaine produces the same approximate degree of respiratory inhibition regardless of the presence of other agents. The effects (if any) of the other agents thus appear to be additive with those of procaine.

DISCUSSION

The present observation that the rates of oxygen uptake in brain tissue are similar in either CO_2–bicarbonate or Tris solutions is in agreement with studies by Elliott and co-workers[26–28] and by Craig[2] which utilized differential manometric methods to examine oxygen uptake in brain cortex slices or homogenates. Measurement of similar respiration rates in differently buffered solutions indicates that the large amount of information on brain cortex respiration in CO_2-free media may be correlated with

TABLE 6. COMPARISON OF EFFECTS OF PROCAINE WITH THOSE OF OTHER AGENTS IN CORTEX AND STRIATUM SLICES*

Brain area slices	No. of slices	Agent added initially	(A) Control Respiration rate (μmoles/hr/g)	(B) After initial agent Respiration rate (μmoles/hr/g)	(C) After 10 mM procaine + 70 μM potassium Respiration rate (μmoles/hr/g)	Effect due to procaine (B minus C) Respiration rate (μmoles/hr/g)	Effect due to procaine plus other agent (A minus C) Respiration rate (μmoles/hr/g)
Cortex	6	None	85·4 ± 5·7		50·9 ± 3·9	34·5 ± 8·5	
Cortex	4	Pentobarbital	91·1 ± 2·5	71·2 ± 9·3	37·5 ± 3·0	33·6 ± 9·8	53·6 ± 4·3
Cortex	5	Caffeine	93·6 ± 7·0	86·9 ± 7·0	52·7 ± 2·6	34·3 ± 5·8	40·7 ± 5·7
Cortex	3	Nicotine	99·1 ± 5·7	99·1 ± 5·7	61·2 ± 8·4	37·9 ± 3·8	37·9 ± 3·8
Striatum	5	None	44·5 ± 3·0		35·8 ± 3·4	8·7 ± 1·7	
Striatum	4	Pentobarbital	57·4 ± 2·3	44·3 ± 1·5	33·6 ± 4·0	10·7 ± 4·5	23·9 ± 5·6
Striatum	5	Caffeine	54·8 ± 2·5	47·3 ± 1·5	37·7 ± 0·9	9·7 ± 0·5	17·1 ± 1·8
Striatum	3	Nicotine	57·6 ± 1·5	57·4 ± 2·2	53·1 ± 4·2	4·3 ± 4·7	4·5 ± 4·7

* Mean values for respiration rates ± S.E.M.

measurements of ion contents and movements in the presence of CO_2. However, addition of stimulatory or inhibitory agents to the incubation solution may induce differing qualitative or quantitative effects on tissue respiration in each of the two solutions. The effects of procaine were not altered by changing the buffered solution, but the concentration of procaine employed had a stronger depressant effect on both stimulated and unstimulated respiration than was reported by Geddes and Quastel.[9] Possibly, this variation in sensitivity may be due to use of different strains of rats or to minor differences in solution composition or pH. The procaine concentration employed (10 mM) is considerably higher than that required (< 1 mM) to abolish the electrically-induced increase in oxygen uptake[12,29] and in ^{22}Na influx.[12]

Pentobarbital inhibited unstimulated respiration by approximately the same amount as has been reported for this agent in experiments employing Warburg apparatus.[30] The potassium-induced increase in respiration was inhibited but not abolished even though the unstimulated respiration was markedly reduced. This is in accord with reports that pentobarbital[30] and other barbiturates[7,8] do not exert a totally selective inhibition of the stimulated respiration. Effects elicited with pentobarbital or caffeine were additive with those of procaine. Since procaine has long been known to exert a strong stabilizing action at the cell membrane,[31] it is likely that pentobarbital and caffeine inhibit tissue respiration by other, possibly more direct, cellular actions. Indeed, other barbiturates are known to have direct effects on cellular metabolism.[12] In contrast to these agents, nicotine did not interfere with cellular metabolism. This lack of action on oxygen utilization by a relatively high concentration of nicotine corresponds to the reported lack of alteration of oxygen uptake by such agents as acetylcholine,[32,33] picrotoxin and strychnine.[34]

Differences between drug-induced actions in either cortex or striatum slices were not extensive. The ratio between respiratory rates in the two slice preparations agrees with values obtained with calf brain slices in Warburg apparatus.[35] The absolute rate of oxygen uptake in cortical slices (about 90 μmoles/hr/g wet weight) is also in agreement with the values observed in the experiments employing Warburg apparatus.[35] Even the small differences observed between some of the control groups could possibly be attributed to experimental temperature variations of about 1°. Considering the numbers of tissues employed in each group, averaged values appeared to be reasonably consistent and stable.

The initial respiratory rate was obtained at an approximate oxygen partial pressure of 600 mm Hg (as compared to 760 mm Hg in Warburg experiments). The similar values for oxygen consumption obtained with Warburg equipment or with the oxygen electrode indicates that diffusion of oxygen through the slices is probably not a rate-limiting process for the uptake of oxygen during the initial portion of the incubation in a balanced medium. However, the diffusion of oxygen may be rate-limiting for K^+-induced stimulation of cortical slices. This possibility is indicated by the smaller effect of added potassium on oxygen uptake in cortex slices than in the less intensely respiring striatum slices (Table 1). In both cases the quantitative effects of added potassium were evident after only a very short interval. This rapid response indicates that the increased respiration is directly related to a high extracellular potassium concentration.[35] A secondary effect due to an increased sodium concentration[36] can be excluded since only a relatively slow increase in sodium concentration is observed[37] after addition of excess potassium ion.

Acknowledgements—We thank Dr. Ronald W. Estabrook for his interest and for generously allowing us use of his oxygen electrode and related equipment for this study. We also gratefully acknowledge the many technical suggestions concerning this technique provided by Dr. Akio Shigematsu.

REFERENCES

1. F. N. CRAIG and H. K. BEECHER, *J. gen. Physiol.* **26,** 473 (1943).
2. F. N. CRAIG, *J. gen. Physiol.* **27,** 325 (1944).
3. S. -C. CHENG and H. WAELSCH, *Science, N.Y.* **136,** 782 (1962).
4. G. SVAETICHIN, K. NEGISHI, R. FATEHCHAND, B. D. DRUJAN and A. SELVÍN DE TESTA, in *Progress in Brain Research* (Eds. E. D. P. DE ROBERTIS and R. CARREA), Vol. 15, p. 243. Elsevier, Amsterdam (1965).
5. R. W. ESTABROOK, in *Methods in Enzymology* (Eds. R. W. ESTABROOK and M. E. PULLMAN), Vol. 10, p. 41. Academic Press, New York (1967).
6. J. H. QUASTEL and A. H. M. WHEATLEY, *Proc. Roy. Soc.* B **112,** 60 (1932).
7. H. MCILWAIN, *Biochem. J.* **53,** 403 (1953).
8. J. J. GHOSH and J. H. QUASTEL, *Nature, Lond.* **174,** 28 (1954).
9. J. C. GEDDES and J. H. QUASTEL, *Anesthesiology* **17,** 666 (1956).
10. J. H. QUASTEL, *Br. med. Bull.* **21,** 49 (1965).
11. H. H. HILLMAN, W. J. CAMPBELL and H. MCILWAIN, *J. Neurochem.* **10,** 325 (1963).
12. S. L. CHAN and J. H. QUASTEL, *Biochem. Pharmac.* **19,** 1071 (1970).
13. C. P. BIANCHI, *J. gen. Physiol.* **44,** 845 (1961).
14. W. G. NAYLOR, *Am. J. Physiol.* **204,** 969 (1963).
15. C. P. BIANCHI, in *Cell Calcium*, p. [59]. Appleton-Century-Crofts, New York (1968).
16. W. Y. CHEUNG, *Biochemistry, N.Y.* **6,** 1069 (1967).
17. W. G. NAYLOR, *Am. J. Physiol.* **205,** 890 (1963).
18. G. B. WEISS, *J. Pharmac. exp. Ther.* **154,** 595 (1966).
19. G. B. WEISS, *J. Pharmac. exp. Ther.* **154,** 605 (1966).
20. G. B. WEISS, *J. Pharmac. exp. Ther.* **163,** 43 (1968).
21. G. B. WEISS, *J. Pharmac. exp. Ther.* **160,** 135 (1968).
22. G. B. WEISS and J. A. ROSECRANS, *Eur. J. Pharmac.* **14,** 130 (1971).
23. G. FRANCK, M. CORNETTE and E. SCHOFFENIELS, *J. Neurochem.* **15,** 843 (1968).
24. L. HERTZ, A. SCHOUSBOE and G. B. WEISS, *Acta physiol. scand.* **79,** 506 (1970).
25. T. ARNFRED, L. HERTZ, L. LOLLE and H. LUND-ANDERSEN, *Expl Brain Res.* **11,** 373 (1970).
26. K. A. C. ELLIOTT and B. LIBET, *J. biol. Chem.* **143,** 227 (1942).
27. M. K. BIRMINGHAM and K. A. C. ELLIOTT, *Fedn Proc.* **7,** 146 (1948).
28. K. A. C. ELLIOTT, *Can. J. Biochem. Physiol.* **33,** 466 (1955).
29. B. M. BOLLARD and H. MCILWAIN, *Biochem. Pharmac.* **2,** 81 (1959).
30. J. L. WEBB and K. A. C. ELLIOTT, *J. Pharmac. exp. Ther.* **103,** 24 (1951).
31. A. M. SHANES, *A. Rev. Pharmac.* **3,** 185 (1963).
32. H. WALLGREN, *Biochem. Pharmac.* **6,** 195 (1961).
33. H. YOSHIDA and J. H. QUASTEL, *Biochim. biophys. Acta* **57,** 67 (1962).
34. J. H. QUASTEL, *Res. Publs Ass. Res. nerv. ment. Dis.* **40,** 57 (1962).
35. L. HERTZ and T. CLAUSEN, *Biochem. J.* **89,** 526 (1963).
36. P. D. SWANSON and H. MCILWAIN, *J. Neurochem.* **12,** 877 (1965).
37. H. LUND-ANDERSEN and L. HERTZ, *Expl Brain Res.* **11,** 199 (1970).