cyclase (Table 2)—IPTBO and *N*-methyl bicuculline were equipotent and picrotoxin was an order of magnitude less potent—and compounds capable of reversing the effects of GABA antagonists in certain tests [9], diazepam and pentobarbitone, raised the level of cyclic AMP production at 1 μM concentration to that produced by 5 μM GABA. The convulsants had no obvious effects on the basal rate of the enzyme, and the anticonvulsants had no effects on the activation by GABA. None of the compounds affected either the basal rate of the ACh-activated rate of the guanylate cyclase. The mechanisms involved are not yet known but are being studied: for example, the elevation of the basal rate of adenylate cyclase by the anticonvulsants may be caused by a GABA-mimetic action, by the release of endogenous GABA, or, perhaps, by an action on the protein inhibitor of high-affinity binding of GABA [10,11]. However, the results are consistent with the possible function of cyclic AMP as a secondary messenger for GABA in the cerebellum, and of cyclic GMP as a secondary messenger for ACh.

The GABA-activated adenylate cyclase might provide a basis for a screening method for potential convulsant and anticonvulsant compounds. However, it must be noted that the cerebellum receives fibres from many parts of the brain [12], and drugs affecting these parts could as a consequence affect the output from the cerebellum; hence such convulsant or anticonvulsant compounds may have no direct effect on the GABA-activated enzyme.

Biology Division,
Chemical Defence Establishment,
Porton Down,
Wiltshire,
SP4 0JQ

D. B. COULT
D. J. HOWELLS

REFERENCES

1. D. B. Coult and D. J. Howells, *Biochem. Pharmac.* **28**, 193 (1979).
2. A. G. Gilman, *Proc. natn. Acad. Sci. U.S.A.* **67**, 305 (1970).
3. B. L. Brown, J. D. M. Albano, R. P. Ekins and A. M. Sgherzi, *Biochem. J.* **121**, 561 (1971).
4. A. L. Steiner, R. E. Wehmann, C. W. Parker and D. M. Kipnis in *Advances in Cyclic Nucleotide Research* (Eds. P. Greengard and G. A. Robinson) Vol. 2, pp. 51–61. Raven Press, New York (1972).
5. M. H. Makman, in *Biochemical Actions of Hormones* (Ed. G. Litwack) Vol. 4, pp. 407–496. Academic Press, London (1977).
6. N. G. Bowery, J. F. Collins and R. G. Hill, *Nature, Lond.* **261**, 601 (1976).
7. G. A. R. Johnston, P. M. Beart, D. R. Curtis, C. J. A. Game, R. M. McCulloch and R. W. McClachlan, *Nature, New Biol.* **240**, 219 (1972).
8. J. C. Eccles, R. F. Schmidt and W. D. Wills, *J. Physiol, Lond.* **168**, 500 (1973).
9. N. G. Bowery and A. Dray, *Br. J. Pharmac.* **63**, 197 (1978).
10. G. Toffano, A. Guidotti and E. Costa, *Proc. natn. Acad. Sci. U.S.A.* **75**, 4024 (1978).
11. A. Guidotti, G. Toffano and E. Costa, *Nature, Lond.* **275**, 553 (1978).
12. H. A. Marke and F. M. Folke, *Synopsis of Neuroanatomy* Chapt. 13. Oxford University Press, New York (1972).

Biochemical Pharmacology, Vol. 28, pp. 2675–2677.
Pergamon Press Ltd. 1979. Printed in Great Britain.

Rotenone and oligomycin-like action of trimebutine on liver mitochondria

(*Received* 5 *February* 1979; *accepted* 22 *March* 1979)

In a previous paper it was shown that papaverine and related compounds inhibit liver mitochondria respiration by a rotenone-like mechanism [1]. This finding raised the question as to whether the biochemical mechanism of some spasmolytic agents might involve significant alterations of mitochondrial functions. We have now reexamined the problem by using trimebutine (phenyl-2-dimethyl-amino-2-n, butanol-trimethoxy-3,4,5 benzoic acid), a drug exhibiting spasmolytic activity, which selectively inhibits the "tonic phase" of intestinal smooth muscle contraction induced by acetylcholine or histamine. Since this compound of the smooth muscle contraction seems to be strictly dependent on aerobic energy [1], an insight into the possible interference of the drug with mitochondrial energy dependent processes can provide useful indications for unravelling the mechanism of action of the drug. In the present communication is reported that trimebutine exhibits on liver mitochondria both a rotenone-like and oligomycin-like action which might be relevant for explaining its pharmacological effects [2].

Methods and results. Rat liver mitochondria were isolated according to Schneider [3]. The amount of protein was determined by the biuret method, as described by Gornall [4]. Oxygen uptake was measured polarographically with a Clark electrode coupled to a Perkin Elmer 56 Recorder. ATPase activity was estimated from pH records [5].

Figure 1A shows that in the presence of NAD-dependent substrates (glutamate plus malate or hydroxybutyrate) trimebutine inhibited oxygen uptake stimulated either by ADP (state 3) or FCCP (uncoupled state). This inhibition is concentration-dependent and it is almost complete at 0.15 mM-trimebutine. The inhibition of oxygen uptake induced by trimebutine was not affected by addition of NAD^+ (results not shown), while it was overcome by menadione (K_3) (Fig. 2). With succinate as substrate trimebutine only slightly affected the rate of respiration in state 4 (results not reported), as well as the rate of respiration released by FCCP (Fig. 1B). These findings clearly indicate that trimebutine exhibits a typical rotenone-like action. The small inhibition of succinate respiration in state 4 or in the presence of FCCP (Fig. 1B) can be interpreted as a slight inhibition of succinic dehydrogenase itself, or of the electron flow through the respiratory chain beyond the rotenone sensitive site.

On the other hand trimebutine inhibited state 3 respiration (Fig. 1B) as well as state 4 → 3 transition induced by ADP with succinate as substrate; FCCP completely relieved such an inhibition (Fig. 3A). This would indicate that trimebutine inhibits ADP phosphorylation with an oligomycin-like mechanism. This assumption is further supported by the results of Fig. 3, which show that trimebutine, as well as oligomycin, did not affect the release of respiration by FCCP. Figure 3B shows that trimebutine, as well as oligomycin, did not affect the release of respiration induced by added Ca^{2+}.

Finally, the oligomycin-like action of trimebutine is also shown by the inhibition of ATP hydrolysis evoked by the uncoupler FCCP (Fig. 4).

Discussion. The results reported in the present paper show

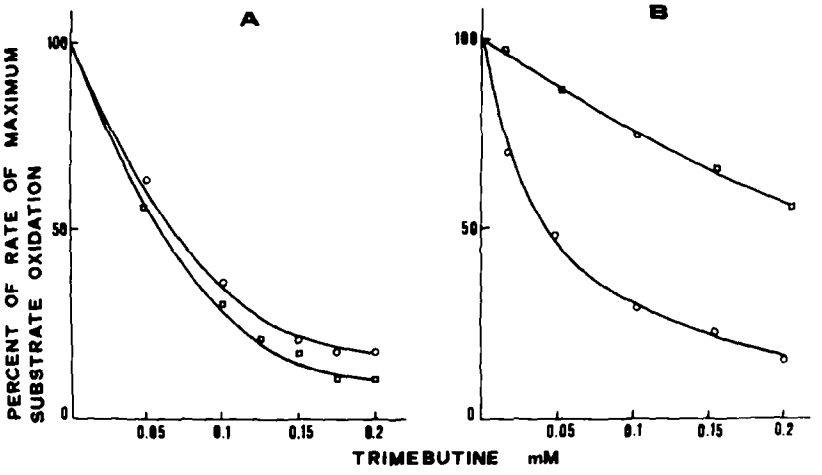

Fig. 1. Effect of trimebutine (TB) on rat liver mitochondria respiration with glutamate (A) and succinate (B) as substrates. 5 mg of mitochondrial protein were added to the following medium: 170 mM sucrose, 10mM-Tris–HCl pH 7.4, 1 mM-Na-phosphate pH 7.4. (A) 10mM-Na-glutamate + 1mM-Na-malate. (B) 10mM-Na-succinate and 1.25μM-rotenone. Final volume 2 ml, temperature 20°. Respiration was stimulated by addition of 1mM ADP (state 3 respiration O- -–O) or 0.8μM-FCCP (uncoupled respiration _|– — ').

that trimebutine exhibits both a rotenone-like and oligomycin-like action. The former action is clearly shown by the inhibition of uncoupled respiration only when NAD-dependent substrates are the electron donors (Fig. 1A). This effect cannot be due to a release of endogenous NAD, because the inhibition of respiration with glutamate plus malate is removed, as in the case of rotenone, by addition of menadione (Fig. 2), while added NAD$^+$ does not have any effect.

The oligomycin-like action of trimebutine is revealed by both the inhibition of state 3 respiration in the presence of succinate, and the inhibition of uncoupler induced ATP hydrolysis (Figs. 3A and 4). As expected, trimebutine, like oligomycin, does not affect the release of respiration induced by Ca^{2+} (Fig. 3B).

These findings strongly support the concept that trimebutine does not affect mitochondria energy linked processes at the first level of energy conservation, i.e. before the entry of P_i and ADP in the coupling sequence. Consequently, the energy deriving from electron transport is diverted from ATP formation towards energy-dependent Ca^{2+} movements.

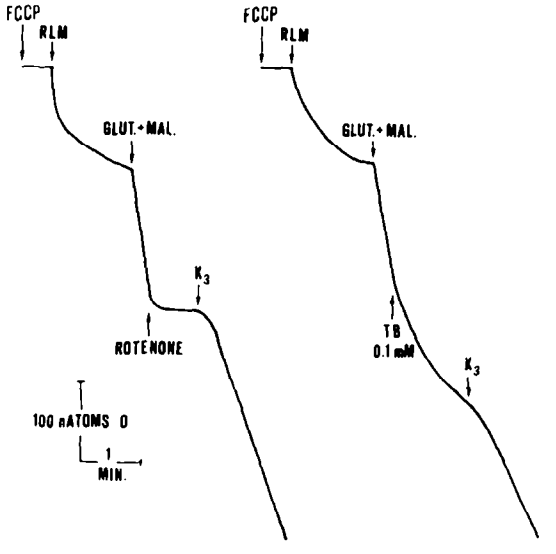

Fig. 2. Release by menadione of inhibition of glutamate oxidation induced by trimebutine. Experimental conditions as in Fig. 1A. Additions: 0.8μM-FCCP, 10mM-Na-glutamate + 1mM-Na-malate, 1.25μM-rotenone, 0.1mM-trimebutine, 0.5mM-menadione (K_3).

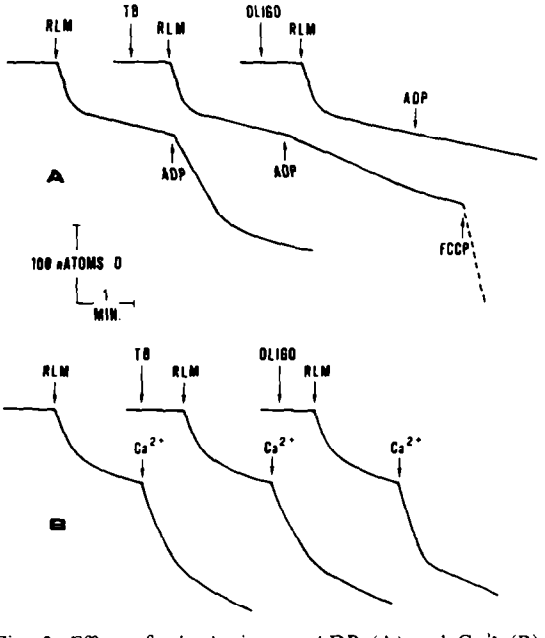

Fig. 3. Effect of trimebutine on ADP (A) and Ca^{2+} (B) respiratory control. 5 mg of mitochondrial protein (RLM) were added to the following medium: 170 mM sucrose, 10mM-Tris–HCl pH 7.4, 10mM-Na-succinate, 1.25μM-rotenone. Final volume 2 ml, temperature 20°. 1mM-Na-phosphate pH 7.4 was present in A. When present 0.2mM-trimebutine, 1μM-oligomycin (oligo). Additions: (A) 0.15mM-ADP, 0.8μM-FCCP; (B) 0.25mM-$CaCl_2$.

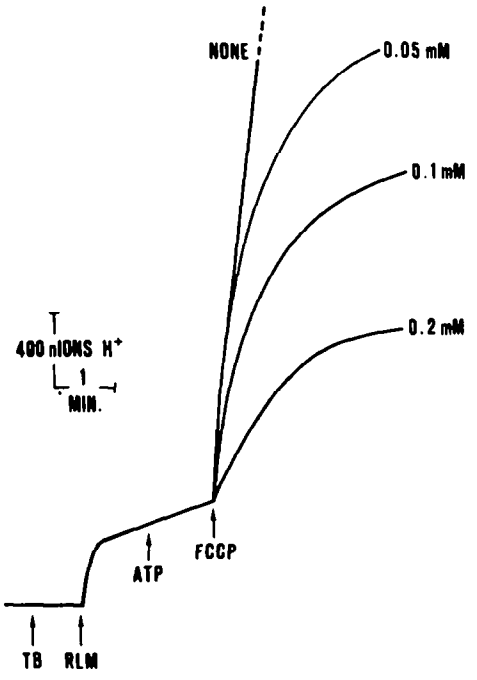

Fig. 4. Effect of trimebutine on mitochondrial ATPase activity stimulated by FCCP. 5 mg of mitochondrial protein (RLM) were added to the following medium: 80mM-KCl, 5mM-Tris–HCl ph 7.4. Final volume 2 ml, temperature 20°. Additions: 0.5mM-ATP, 0.8μM-FCCP.

This oligomycin-like action of trimebutine by inhibiting ATP formation in favour of other energy-dependent processes, like Ca^{2+} transport, can offer a basis for a further insight into the mechanism by which this kind of drug exhibits its pharmacological effects [1, 2].

In the light of current knowledges it is difficult to correlate the rotenone-like action of trimebutine with its oligomycin-like effect. The two actions seem to be quite independent. To our knowledge no other compound shares this peculiar feature.

Addendum: Trimebutine was kindly supplied by SIGMA-TAU (Roma, Italy)

Centro per lo Studio della Fisiologia Mitocondriale CNR, 35100 *Padova, Italy*

D. SILIPRANDI
A. TONINELLO

Institute of Pharmacology, University of Padua 35100 *Padova, Italy*

S. GOBBATO

REFERENCES

1. R. Santi, M. Ferrari and A. R. Contessa, *Biochem. Pharmac.* **13**, 153 (1964).
2. S. Gobbato, R. Padrini, P. Finotto and G. Cargnelli *Farmacol. Ter. Riv. It.*, (in press).
3. W. C. Schneider in W. W. Umbreit, R. Burris and J. D. Stauffer, *Manometric techniques*, p. 188. Burgess Minneapolis (1956).
4. A. G. Gornall, C. J. Bardawill and M. M. David, *J. biol. Chem.* **177**, 751 (1949).
5. M. Nishimura, T. Ito and B. Chance, *Biochim. biophys. Acta* **59**, 177 (1962).

Biochemical Pharmacology, Vol. 28, pp. 2677–2679
Pergamon Press Ltd. 1979. Printed in Great Britain.

0006-2952/79/0901–2677 $02.00/0

Effect of ethanol and stress on gamma-aminobutyric acid and guanosine 3′, 5′-monophosphate levels in the rat brain

(*Received* 14 *December* 1978; *accepted* 8 *March* 1979)

The acute effect of ethanol on gamma-aminobutyric acid (GABA) levels in the brain has been studied by a number of investigators [1–18]. However, the published results are contradictory because increases [1–9], no change [7–16], and decreases [6, 16–18] of GABA levels in the brain were reported. Several factors have been investigated as possible causes of these divergent results. These include differences in the methods used for tissue fixation and GABA determination [1, 8], strain and species differences [7, 10], and different nutritional states [8, 11, 16]. However, none of these factors explains satisfactorily why the observed effects of ethanol on GABA levels have been so variable.

Discovery of a reciprocal relationship between levels of guanosine 3′, 5′-monophosphate (cyclic GMP) and GABA [19] resulted in a renewed interest in the role of GABA as a neurotransmitter. Since acute ethanol administration causes a pronounced decrease in cyclic GMP levels [20–22] and GABA levels are decreased during ethanol withdrawal [23, 24], we decided to re-investigate the effect of acute ethanol administration on GABA levels. We found in one experiment that ethanol produced a dose-dependent decrease of GABA in the cerebellum and pons-medulla oblongata [20]. However, although experimental results were consistent when a single investigator repeated an experiment, they varied when experiments were repeated in our laboratory by various investigators even though the same animal strain, dose of ethanol, and method of GABA determination were used. This variability might be caused by subtle differences in handling of animals before they are killed, thus inducing variable degrees of stress. Therefore, we investigated the interaction between stress and the effects of ethanol on GABA and cyclic GMP levels.

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), weighing 200–250 g, were used for all experiments, and 4 g/kg of ethanol (16.7% v/v) (USP) was administered in a volume of 3 ml/100 g *per os* by intubation. It had been found in previous experiments that this dose produces an inability to stay on an inclined plane in 65 per