1 This research was supported by a grant from the Deutsche Forschungsgemeinschaft (Ja/185/6).
2 D.T. Witiak and M.W. Whitehouse, Biochem. Pharmac. *18*, 971 (1969).
3 E.M. Sellers and J. Koch-Weser, Pharmac. Res. Commun. *7*, 331 (1975).
4 W.E. Müller and U. Wollert, Molec. Pharmac. *11*, 52 (1975).
5 A. Yacobi and G. Levy, J. Pharmacokin. Biopharm. *5*, 123 (1977).
6 W.E. Müller and U. Wollert, Res. Commun. chem. Path. Pharmac. *9*, 413 (1974).
7 W. Schmidt and E. Jähnchen, J. Pharm. Pharmac. *29*, 266 (1977).
8 E.M. Sellers, M.L. Lang-Sellers and J. Koch-Weser, J. Biochem. Pharmac. *26*, 2445 (1977).
9 N.A. Brown, E. Jähnchen, W.E. Müller and U. Wollert, Molec. Pharmac. *13*, 70 (1977).
10 E. Jähnchen, T. Meinertz, H.J. Gilfrich, U. Groth and A. Martini, Clin. Pharmac. Ther. *20*, 342 (1976).
11 J.B. West and K. Link, J. heterocycl. Chem. *2*, 93 (1965).
12 R.A. O'Reilly, Clin. Pharmac. Ther. *16*, 348 (1974).
13 A. Breckenridge, M. Orme, H. Wesseling and J.R. Lewis, Clin. Pharmac. Ther. *15*, 424 (1974).
14 D.S. Hewick and J. McEwen, J. Pharm. Pharmac. *25*, 458 (1973).
15 A. Breckenridge and M. Orme, Life Sci. *11*, 337 (1972).
16 A. Yacobi and G. Levy, J. Pharmacokin. Biopharm. *2*, 239 (1974).

GABA-mimetic action of etomidate

R.H. Evans and R.G. Hill[1]

Department of Pharmacology, Medical School, University of Bristol, Bristol BS8 1TD (England), 16 March 1978

Summary. A comparison of antagonism by bicuculline or strychnine of the effects of GABA or etomidate on rat isolated superior cervical ganglia, frog isolated hemisected spinal cords and rat central neurones in vivo indicates that etomidate has GABA-mimetic actions.

Etomidate is a novel short acting, non-barbiturate hypnotic drug of high potency and low toxicity which is used in the induction of anaesthesia. Etomidate is of particular interest to the pharmacologist because it has 2 optical isomers, the (+)-form being considerably more potent than the (−)-form[2].

We have examined the effects of etomidate on rat neurones in vivo and also on the isolated superior cervical ganglion of the rat[3], the isolated hemisected spinal cord of the immature rat[4] and the isolated hemisected spinal cord of the frog[5]. In all preparations the effects of etomidate were depressed specifically by GABA antagonists, suggesting that this drug produces its central depressant action by mimicking the action of the inhibitory neurotransmitter.

Figure 1 shows the depolarizing effects of carbachol, GABA, etomidate and pentobarbitone recorded from the postganglionic nerve of the isolated superior cervical ganglion of the rat. Carbachol, GABA or (+) etomidate produced dose dependent depolarizations of the ganglion cells. Similar responses were recorded also from dorsal roots of the isolated immature rat spinal cord. Responses to (+) etomidate resembled those to pentobarbitone and were slower in onset and of longer duration than those to GABA. Responses produced by GABA, etomidate or pentobarbitone, but not carbachol, were antagonised by bicuculline. Responses to GABA or the 2 depressant drugs were unaffected by hexamethonium.

Figure 2 shows potentials recorded from dorsal and ventral roots of isolated frog spinal cords. GABA or (+) etomidate produced depolarization of primary afferent fibres and hyperpolarization of motoneurones. As with the superior cervical ganglion and rat spinal cord, the responses to etomidate were greatly prolonged in comparison to those of GABA. Bicuculline (100 μM) depressed responses to GABA or (+) etomidate and similar effects were observed with picrotoxin (50 μM). Strychnine has been shown to antagonize hyperpolarizations of frog motoneurones produced by β-alanine, taurine or glycine, but not those produced by GABA[6,7]. Figure 2, B shows that hyperpolarizations of frog motoneurones produced by (+) etomidate or GABA were unaffected by strychnine (1 μM), whereas responses to taurine were abolished. Each antagonist (bicuculline, picrotoxin or strychnine) was tested against reponses produced by etomidate and GABA or taurine on 2 preparations; complete recovery of frog spinal cord preparations from the antagonists was normally not sought since this took 12 h or longer.

Dose response plots for depolarization of frog primary afferent terminals produced by (+) or (−)etomidate and recorded from a dorsal root are shown in figure 2, C. The (+) isomer was 20 times more potent than the (−) isomer. Responses to the (−) isomer were antagonized also by bicuculline. These in vitro effects of (+) etomidate occur at concentrations similar to those measured in brain (5–30 μM) during behavioural and hypnotic effects[8].

(+) Etomidate, applied microiontophoretically to 67 glutamate excited neurones in the caudal medullary reticular formation (n. reticularis ventralis), produced depression of all of them. When the depressant actions of GABA, glycine and (+) etomidate were compared it was found that the mean currents necessary to produce the same level of depression were similar for applications of glycine or (+) etomidate whereas slightly higher currents were required for applications of GABA. On 13 neurones which were depressed by both GABA and (+) etomidate, iontophoretically applied bicuculline methobromide antagonized the action of GABA on all of these cells and that of (+) etomidate on 12 of them. An example of such an experiment is shown in figure 3. On 5 similar neurones strychnine was found to reverse the depressant action of glycine but not that of (+) etomidate.

Thus, both in vivo and in vitro experiments show that responses to etomidate are specifically antagonized by known GABA antagonists, which indicates that etomidate produces its central depressant effects by a GABA-mimetic action. Pentobarbitone has also been shown to have GABA-mimetic actions[9,10], and it is interesting that the relative GABA-mimetic potency of (+) etomidate and pentobarbitone, indicated in figure 2, approximates to the relative brain levels associated with the hypnotic effects of these 2 drugs[8,11].

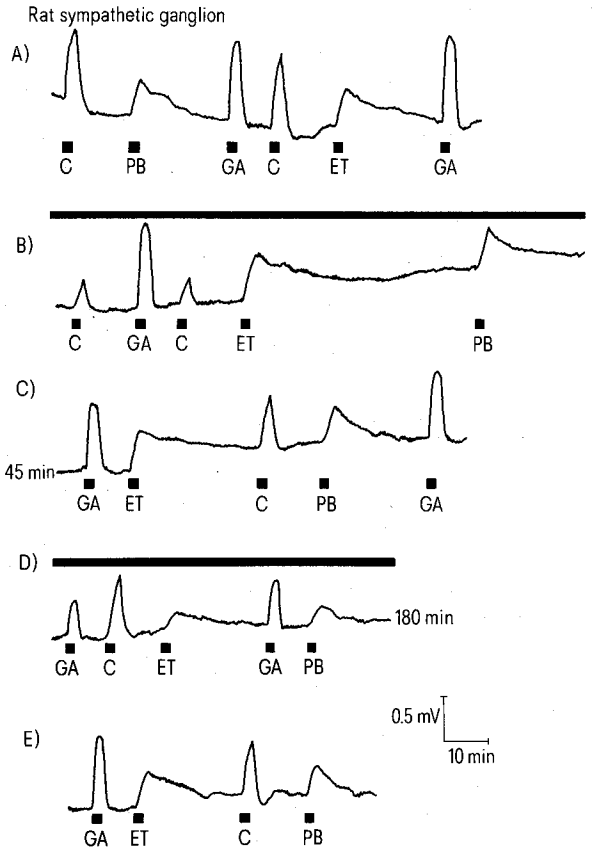

Fig. 1. Continuous recording from postganglionic neurones of a rat isolated superior cervical ganglion. Polarity was recorded between 2 electrodes placed in contact with the ganglion body and the distal end of the postganglionic nerve. Upward deflection on the record indicates an increase in negativity of the ganglion body corresponding to depolarization of sympathetic neurones. The ganglion was maintained at 20 °C and superfused with Ringer solution at a rate of 1 ml/min. The following drugs were dissolved in 2 ml of Ringer solution and introduced as indicated. Carbachol 20 µM (C), pentobarbitone 250 µM (PB), GABA 5 µM (GA) and (+) etomidate 25 µM (ET). The bar above B indicates the introduction of hexamethonium bromide 250 µM and the bar above D, introduction of bicuculline 5 µM.

These GABA-mimetic actions could be caused either directly by an agonist action of the drug on GABA receptors or indirectly via release of, or potentiation[12-14] of, the effects of endogenous GABA. An indirect GABA-mimetic action involving endogenous GABA would seem unlikely because, in the present study, pentobarbitone and etomidate had similar effects on both the rat isolated superior cervical ganglion and the rat isolated spinal cord. The ganglion unlike the spinal cord does not possess GABA containing nerve terminals thus the distribution and release of endogenous GABA in these 2 tissues would be quite different.

Etomidate was consistently less potent than GABA in evoking depolarizations of sympathetic neurones (figure 1) or primary afferent terminals of the rat spinal cord. The true molar potency at receptors, of etomidate compared to GABA would be difficult to determine because of the operation of removal systems for GABA. However, the persistence of etomidate and similar drugs in the vicinity of GABA receptors may be more important than the potency relative to GABA in determining the in vivo depressant action.

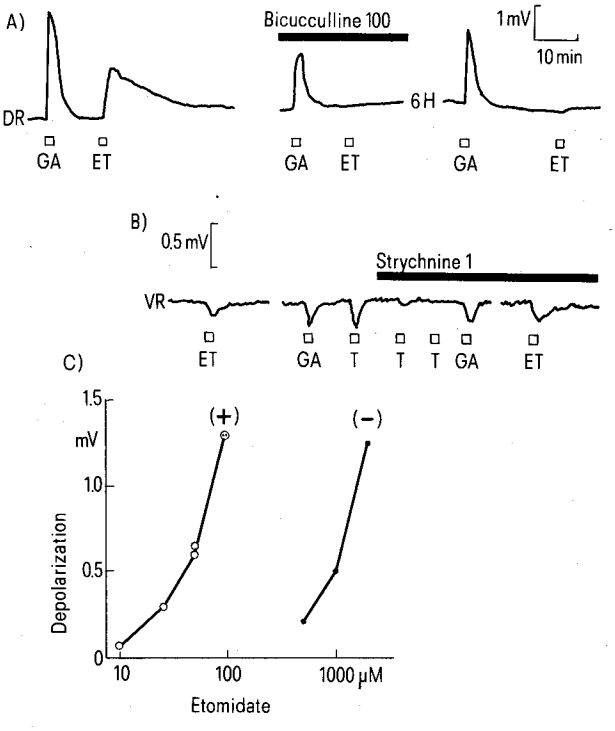

Fig. 2. Recordings from dorsal or ventral roots of 3 isolated hemisected spinal cords of *R. temporaria*. Upward deflection indicates increased positivity of the distal end of a root corresponding to depolarization of afferent fibres (DR) and motoneurones (VR). The hemicords were maintained at 12 °C and the Ringer solution contained tetrodotoxin 0.1 µM to abolish indirect actions; other details as for figure 1. *A* Dorsal root record showing effect of bicuculline 100 µM. GABA 2 mM, (+) etomidate 50 µM. *B* Ventral root record showing effect of strychnine 1 µM. (+) Etomidate 50 µM, GABA 50 µM, taurine 50 µM (T). *C* Comparison of potency of (+) and (−) etomidate. Primary afferent depolarizations recorded as in A.

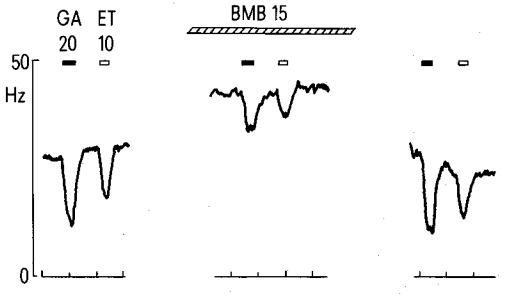

Fig. 3. Analogue ratemeter records of the firing of a single reticular formation neurone in the halothane anaesthetized rat; abscissa, time in minutes. Iontophoretic application of GABA (GA, filled bar) with a current of 20 nA depressed the firing rate of this neurone as did a similar application of (+) etomidate (ETO, open bar). 4 min after the start of a continuous application of bicuculline methobromide (BMB, hatched bar) with a current of 15 nA the effectiveness of both GABA and etomidate applications was reduced and 2 min after switching off the bicuculline methobromide control responses were obtained to both GABA and etomidate. The increase in firing rate during bicuculline methobromide is independent of the antagonist action of this compound[15]. Before BMB was applied GABA and (+) etomidate respectively produced a 61% and 42% depression of firing. During the BMB these values were reduced to 19% and 12% depression and on recovery values of 60% and 50% depression were obtained. The drug solutions placed in the electrodes were as follows; etomidate 0.2 M nitrate salt in distilled water, GABA 0.2 M, pH 3.5, bicuculline methobromide 5 mM in 150 mM NaCl pH 3.5.

1 Acknowledgments. This work was supported by the Medical Research Council and etomidate was provided by Janssen Pharmaceutica.
2 P.A.J. Janssen, C.J.E. Niemegeers, K.M.I. Schellekens and F.M. Lenaerts, Arzneimittel-Forsch. *21*, 1234 (1971).
3 N.G. Bowery and D.A. Brown, Br. J. Pharmac. *50*, 205 (1974).
4 M. Otsuka and S. Konishi, Nature *252*, 733 (1974).
5 D.R. Curtis, J.W. Phillis and J.C. Watkins, Br. J. Pharmac. *16*, 262 (1961).
6 R.H. Evans and J.C. Watkins, Br. J. Pharmac. *55*, 519 (1975).
7 R.H. Evans, A.A. Francis and J.C. Watkins, Brain Res. *118*, 395 (1976).
8 J.J.P. Heykants, W.E.G. Meuldermans, L.J.M. Michiels, P.J. Lewi and P.A.J. Janssen, Archs. int. Pharmacodyn. *216*, 113 (1975).
9 R.A. Nicoll, Brain Res. *96*, 119 (1975).
10 R.A. Nicoll, Proc. nat. Acad. Sci. USA *72*, 1460 (1975).
11 C.D. Richards, J. Physiol., Lond. *227*, 749 (1972).
12 B.R. Ransom and J.L. Barker, Brain Res. *114*, 530 (1976).
13 D.R. Curtis and D. Lodge, J. Physiol., Lond. *272*, 48P. (1977).
14 R.H. Evans, J. Physiol., Lond. *272*, 49P. (1977).
15 J.F. Collins and R.G. Hill, Nature *249*, 845 (1974).

Histological changes in thyroid of rat under the acute exposure of O-chloro-benzylidine malononitrile

A.R. Chowdhury, M.B. Deshmukh, C.D. Raghuveeran, A.B. Nashikkar and A.K. Chatterjee

Defence Research and Development Establishment, Toxicology Division, Gwalior-474002 (India), 1 March 1978

Summary. This communication describes histological changes in the thyroid gland of rats under the acute stress of O-chlorobenzylidene malononitrile (CS) (10 mg/kg and 20 mg/kg). It has been observed that CS, when injected, causes histological changes in the thyroid of varying degrees, depending on the dose used.

O-Chlorobenzylidene malononitrile (CS) was originally developed as a riot control agent (Lacrimator)[2]. Recently Chowdhury et al.[3] had reported the changes in histology and cytometry of the adrenals of rats after i.p. administration of sublethal doses of O-chlorobenzylidene malononitrile (CS). The present communication deals with the histological changes of the thyroid of rats on the same treatment with CS.

Materials and methods. CS was synthesized according to standard methods[4]. 30 female albino rats (180±5 g) from the DRDE colony were used in the experiment. The animals were divided equally into 3 groups. The 1st group (group A) was the control, the 2nd and 3rd groups were injected with a solution of CS in olive oil at a dosages of 10 mg/kg (i.p.) (group B) and 20 mg/kg (i.p.) (group C) respectively for 10 days. The control animals were injected with equivalent amount of olive oil daily for 10 days. The doses were 25% and 50% of the LD_{50}[5] for female rats (LD_{50} = 40 mg CS/kg i.p.). On the 11th day, the animals were sacrificed by cervical dislocation. The thyroid was dissected out and fixed in Bouin's fluid for routine histological processes.

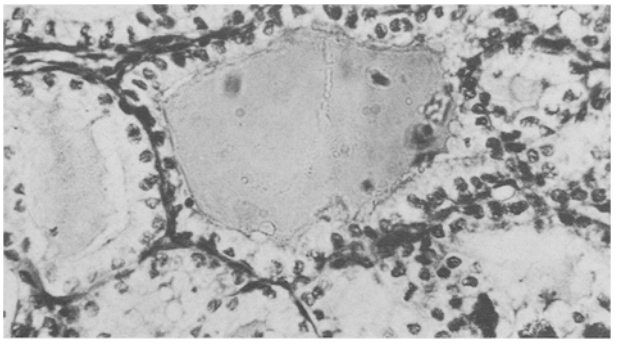

Fig. 1. Normal histological feature of thyroid gland of rat. × 200.

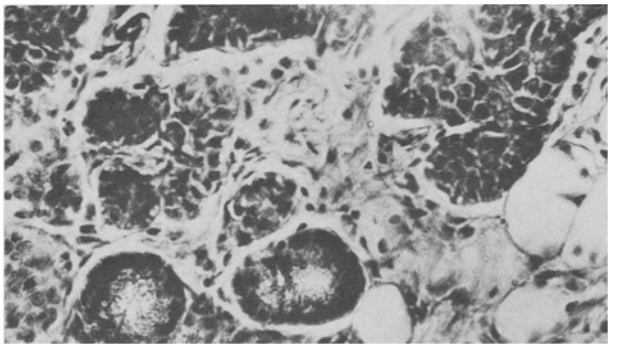

Fig. 2. 20 mg/kg CS shows the complete degeneration of thyroid gland. × 200.

Result and discussion. The histological pattern of the thyroids showed an orderly arrangement of thyroid follicle with an oval nucleus (figure 1) in the control group. After the administration of CS at a dosage of 10 mg/kg, mild degenerative changes were noticed in the thyroid follicle. Follicular epithelium and nuclei in this dosage were highly hyperproliferative, and most of the follicles were filled up with a lower amount of thyroglobulin; the nuclei were vacuolated, but at the dosage of 20 mg/kg, there was complete degeneration of thyroid follicle and in addition calciolysis of the cellular material (figure 2) was observed. Histological changes of the thyroid during acute and chronic stress have been discussed by Brown-Grant et al.[6]. It has been observed by many workers[7] that physical stresses, such as haemorrhage, trauma and the injection of irritating substances, induce a prompt and prolonged inhibition of thyroid secretion, presumably as a consequence of diminished release of TSH. The degenerative changes in the thyroid gland recorded in this paper might also be due to continuous inhibition of the secretion of TSH leading to the atrophy of the thyroid follicle; however, a direct toxic action on the thyroid cannot be ruled out.

1 The authors are thankful to Dr P.K. Ramachandran, Defence Research and Development Establishment, Gwalior for his sustained interest and critical suggestions in the course of this study. We are also thankful to Mr H.K. Taneja for microphotography.
2 M. Meselson, Bull. atom. Scient. *16*, 17 (1971).
3 A.R. Chowdhury, M.B. Deshmukh, A.B. Nashikkar, C.D. Raghuveeran and A.K. Chatterjee, Experientia *34*, 494 (1978).
4 B.B. Corson and W.J. Sloughton, J. Am. chem. Soc. *50*, 2825 (1928).
5 L.C. Miller and M.L. Tainter, Proc. Soc. exp. Biol. Med. *57*, 261 (1944).
6 K. Brown-Grant, G.W. Harris and S. Reichlin, J. Physiol. *126*, 29 (1954).
7 C.D. Turner, in: General Endocrinology, 3rd ed. p. 221, W.B. Saunders Company, Philadelphia and London 1964.