change produced by the concentration of GABA, A.

$$\Delta g = \frac{\Delta g_{max}}{1 + \left(\frac{K_{\frac{1}{2}}}{A}\right)^{n_H}} \quad (1)$$

$K_{\frac{1}{2}}$ is the concentration of GABA producing the half maximum conductance change, Δg_{max} is the maximum conductance change, and n_H is the Hill coefficient. A least squares estimate of the values of n_H, $K_{\frac{1}{2}}$ and Δg_{max} was made using an iterative procedure for each of the 6 bags. The size of the responses did not appear to depend upon the location of the bags. The mean ± s.e. mean of n_H was 3.1 ± 0.6 (n = 6). The mean ± s.e. mean of $K_{\frac{1}{2}}$ was 18 ± 3 μM (n = 6). The mean ± s.e. mean of Δg_{max} was 4.5 ± 0.9 M Ω^{-1} (n = 6).

It was also possible to estimate the reversal potentials of the GABA responses by extrapolation. The mean ± s.e. mean was 29.5 ± 2 mv (n = 7) more negative than the resting potential at 10^{-5} M GABA.

References

DEL CASTILLO, J., DE MELLO, W.C., & MORALES, T. (1964a). Inhibitory action of γ-aminobutyric acid (GABA) on Ascaris muscle. *Experientia*, **20**, 141–145.

DEL CASTILLO, J., DE MELLO, W. C. & MORALES, T. (1964b). Mechanism of the paralysing action of piperazine on Ascaris muscle. *Br. J. Pharmac.*, **22**, 463–477.

Induction of analgesia and morphine potentiation by irreversible inhibitors of GABA-transaminase

W.R. BUCKETT

Centre de Recherche Merrell International, 16, rue d'Ankara 67084-Strasbourg Cedex, France

Attempts have been made to ascribe a role for GABA in analgesia, but the results have been controversial. For example, the GABA agonist muscimol enhances morphine analgesia (Biggio, Della Bella, Frigeni & Guidotti, 1977), whereas the GABA-transaminase inhibitor aminooxyacetic acid has been reported to antagonize morphine analgesia (Ho, Loh & Way, 1976). The recent availability of two irreversible inhibitors of GABA-transaminase, γ-vinyl GABA (GVG; DL-4-aminohex-5-enoic acid; RMI 71754) and γ-acetylenic GABA (GAG; DL-4-aminohex-5-ynoic acid; RMI 71645) led to this study of their analgesic properties, their effects upon morphine analgesia and their utilization during morphine-withdrawal to detect any change in sensitivity of the GABA system.

Analgesia was tested using groups of ten CD_1 mice (20–25 g) on a 52°C hot-plate. GVG (ED_{50} 770 (670–870) mg/kg i.p.) and GAG (ED_{50} 51 (46–56) mg/kg i.p.) were active, but at higher doses than morphine HCl (ED_{50} 6.9 (6.1–7.7) mg/kg s.c.). Analgesia was maximal 4 to 6 h after GVG or GAG (ED_{50} dose i.p.), correlating with the rise in mouse brain GABA levels (Jung *et al.*, 1977a, b). Analgesia could be demonstrated with the (+)-isomer of GVG, the form active as a GABA-transaminase inhibitor; none was detectable after the inactive (−)-isomer of GVG up to 800 mg/kg i.p. GVG and GAG-induced analgesia was not preventable by naloxone HCl (1 mg/kg), nor did these compounds inhibit (up to 10^{-4} M) the contractions of the isolated transmurally-stimulated guinea pig ileum, indicating lack of opioid involvement. In the rat, analgesia was shown using the tail-stimulation test (Hoffmeister, 1968) using groups of six male Sprague–Dawley rats (140–170 g). Although GVG (ED_{50} 1100 (960–1240) mg/kg i.p.) and GAG (ED_{50} 61 (50–72) mg/kg i.p.) were less active than morphine HCl (ED_{50} 1.60 (1.40–1.90) mg/kg s.c.), they also inhibited both vocalization and vocalization after-discharge.

The analgesic activity of morphine HCl in mice on the 56°C hot-plate was potentiated by a factor of three after GVG (800 mg/kg i.p.), with GVG itself being inactive. Lower doses of GVG did not potentiate morphine, suggesting a large increase in GABA levels (Jung *et al.*, 1977b) is necessary for this action. In rats withdrawn from morphine (60–840 mg/kg i.p. through 14 days), the hypothermia elicited by GVG (800 mg/kg i.p.) and GAG (100 mg/kg i.p.) did not differ significantly from that in controls, indicating that no change in sensitivity in the GABA system occurs during withdrawal.

It is concluded that the administration of GVG and GAG can result in analgesia, with a temporal relationship between analgesia and increased brain GABA levels. Only the GVG isomer which is active as a GABA-transaminase inhibitor has analgesic properties. There is enhancement of morphine analgesia by elevated GABA levels, although changes in sensitivity of the GABA system on morphine-withdrawal are not evident. These results support a causal relationship between increased brain GABA levels and analgesia, although the active sites remain to be explored.

References

BIGGIO, G., DELLA BELLA, D., FRIGENI, V. & GUIDOTTI, A. (1977). Potentiation of morphine analgesia by muscimol. *Neuropharmac.*, **16**, 149–150.

HO, I.K., LOH, H.H. & WAY, E.L. (1976). Pharmacological manipulation of gamma-aminobutyric acid (GABA) in morphine analgesia, tolerance and physical dependence. *Life Sci.*, **18**, 1111–1124.

HOFFMEISTER, F. (1968). Effects of psychotropic drugs on pain. In: *Pain.*, Ed. Soulairac, A., Cahn, J. & Charpentier, J., London: Academic Press, pp. 309–319.

JUNG, M.J., LIPPERT, B., METCALF, B.W., SCHECHTER, P.J., BÖHLEN, P. & SJOERDSMA, A. (1977a). The effect of 4-amino hex-5-ynoic acid (γ-acetylenic GABA, γ-ethynyl GABA) a catalytic inhibitor of GABA transaminase, on brain GABA metabolism *in vivo*. *J. Neurochem.*, **28**, 717–723.

JUNG, M.J., LIPPERT, B., METCALF, B.W., BÖHLEN, P. & SCHECHTER, P.J. (1977b). γ-Vinyl GABA (4-amino-hex-5-enoic acid), a new selective irreversible inhibitor of GABA-T; effects on brain GABA metabolism in mice. *J. Neurochem.*, **29**, 797–802.

Some neurochemical effects of chronic oral administration of ethanolamine *O*-sulphate to rats

A. FLETCHER & L.J. FOWLER
(introduced by M.A. SIMMONDS)

Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX

Ethanolamine *O*-sulphate (EOS), a specific irreversible inhibitor of GABA-transaminase (GABA-T) (Fowler & John, 1972) has been widely used to raise brain GABA levels in experimental animals. In most instances EOS has been administered directly into the CSF on the assumption that peripheral administration is ineffective. However, when administered in a high dose subcutaneously (Leach & Walker, 1977) EOS was found to effectively inhibit GABA-T and elevate brain GABA concentrations. In this report we investigate oral administration.

Wistar rats (120–200 g) of either sex were given either normal drinking water (controls) or a dilute solution of EOS in distilled water. Daily liquid consumption was measured. The effect of various concentrations of EOS on brain GABA-T (Salvador & Albers, 1959) and GABA levels (Sutton & Simmonds, 1974) were measured after 12 days. Brain homogenates were prepared in ice-cold distilled water within 40 s of decapitation. The time-course of the effects of a fixed dose (5 mg/ml) were measured in a separate experiment.

The results in Table 1 show that oral EOS inhibited GABA-T in a dose-dependent manner. However, the increase in GABA was not as clearly dose-dependent. Similarly, the fixed dose (5 mg/ml) of EOS caused progressively greater inhibition of GABA-T up to 14 days (the maximum time period studied). After 1 day on 5 mg/ml EOS, GABA-T activity was 86.2 ± 3.9% of controls ($P < 0.02$, $n = 5$) and at 14 days was 22.6 ± 1.88% of controls ($P < 0.001$, $n = 3$). At these times GABA levels were 171.4 ± 16.5% ($P < 0.01$, $n = 4$) and 178.9 ± 19.5% ($P < 0.001$, $n = 3$) of controls, respectively.

It is possible that when GABA-T is chronically inhibited a compensatory mechanism operates to prevent GABA levels from becoming excessively high. Preliminary experiments suggest that glutamate

Table 1 The effect of various concentrations of EOS on brain GABA and GABA-T after 12 days

EOS (mg/ml)	*n*	*GABA-T*	*GABA*	*Mean daily water consumption per rat (ml)*
Controls	5	100	100	23.4
0.75	5	73.5 ± 3.6*	126.5 ± 8.0****	21.0
1.50	5	48.3 ± 2.7*	143.3 ± 11.0***	21.3
3.00	5	38.7 ± 2.8*	188.3 ± 2.8*	21.5
6.00	3	23.1 ± 3.0*	149.8 ± 7.5**	17.9
10.0	3	16.5 ± 0.2*	171.0 ± 9.3**	13.0

Values are percentage control ± s.e. Control GABA-T activity 115.3 ± 1.8 μmol g^{-1} h^{-1}. Control GABA levels 3.95 ± 0.23 μmol/g. **** $P < 0.05$, *** $P < 0.02$, ** $P < 0.01$, * $P < 0.001$, compared with controls (Student's *t*-test).