8–10, when changes in vagina, like loosening and spotting and depression of mammary glands, were observed. The compound was also administered at the dose level of 60 mg/kg b.wt on days 10, 12, 14 and 16 of pregnancy. Those showing signs of abortion were laparotomized 4 days after drugging, while those without any signs of abortion were allowed to go to term and the litters were observed for any morphological deformities. In both the above cases, the control animals were treated with vehicle only.

The results indicate that the compound exerted 100% abortifacient activity at the dose level of 60 mg/kg b.wt when fed on day 6 or 7 of pregnancy. Successive lower doses showed lower percentages of activity. The dose response relationship is represented by the regression line (figure) which has been plotted by using the equation $y = \bar{y} + b(x - \bar{x})$[5] where the calculated value for b was 2.01 and the correlation coefficient was 0.9986. Only 25% and 20% of abortifacient activity were recorded on days 10 and 12, respectively, while the compound had no effect on days 14 and 16 (table). Laparotomy of the aborting females in both the above cases revealed empty uterine lumen or lumen with degenerating fetus, while the control animals exhibited intact implantation sites with fetus. No toxic effect was observed at the dose levels used and no deformities were found upto the F_1 generation.

1 The authors wish to thank Dr S.C. Pakrashi and his associate Mr P.P. Ghosh Dastidar for the compound. Thanks are also due to the Director General of Indian Council of Medical Research for granting a fellowship to one of them (C.S.).

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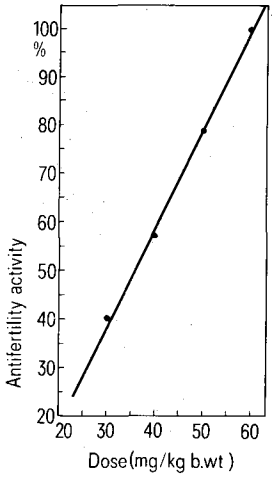
Effect of methyl ester of aristolic acid at the post implantation stages of pregnancy in mice

No. of mice used	Day of administration	Dose in mg/kg b.wt	Mice showing antifertility activity (%)
Control,5,5,5,5	10,12,14,16	Vehicle	0.0
8	10	60	25.0
5	12	60	20.0
8	14	60	0.0
8	16	60	0.0



Regression line representing the relation between dose of the compound and the percentage of antifertility effect. Circles represent experimental values. The number of animals used for each of the experimental and the control group was 10.

Isoguvacine, isonipecotic acid, muscimol and N-methyl isoguvacine on the GABA receptor in rat sympathetic ganglia

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Summary. The GABA-mimetic activities of 4 analogues muscimol, isonipecotic acid, isoguvacine and N-methyl isoguvacine have been examined at the GABA receptor in the rat isolated superior cervical ganglion. The depolarizing action of all 4 analogues could be selectively antagonized by bicuculline methochloride and isopropyl bicyclophosphate. Muscimol was the only analogue more potent than GABA (molar potency ratio = 5.08±0.707). The potency of isoguvacine was 0.23±0.026 and isonipecotic acid 0.011±0.0028. N-methyl isoguvacine was < 0.001 GABA.

The discovery of substances which mimic the action of the mammalian neurotransmitter γ-aminobutyric acid (GABA) at its central receptors may be important not only for understanding the characteristics of the receptors but also for obtaining potentially-useful therapeutic agents[1]. Krogsgaard-Larsen et al.[2] have recently reported that the conformationally-restricted analogue of GABA, isoguvacine, is a potent agonist at GABA receptors on feline spinal interneurones. Its activity was comparable with that of another analogue, muscimol, which has been reported to be more potent than GABA not only on spinal interneurones[3] but also as a displacement ligand in receptor binding studies with ^{3}H-GABA[4,5]. The relative molar potencies of receptor agonists can only be determined where their concentrations in the vicinity of the receptor are known. In iontophoretic studies concentrations are not easily obtained and comparison is more usually based on the currents required to expel sufficient analogue from the micropipette to depress the cell firing rate by the same amount as GABA.

In the present study therefore, we have compared the potencies of isoguvacine and its saturated analogue, isonipecotic acid, muscimol, and N-methyl isoguvacine

(figure 1) on the GABA receptor in the isolated superior cervical ganglion of the rat where concentrations are known. Neurones in this sympathetic ganglion possess receptors for GABA[6] which when activated produce an increase in membrane conductance[7]. The magnitude of the resulting depolarization is concentration-dependent and can be detected on the ganglion surface. The characteristics of the ganglionic receptor are comparable with those of central GABA receptors[6] indicating that this tissue provides a simple system in which the potency of potential GABA analogues can be determined.

Materials and methods. Ganglia excised from anaesthetized (urethane 1.4 g/kg) Wistar rats were desheathed and superfused with Krebs-Henseleit solution as described by Brown and Marsh[8]. The ganglion surface potential measured with respect to the post-ganglionic trunk was continuously monitored using a Smiths Servoscribe Is potentiometric recorder. Agonists were dissolved in the superfusion fluid and applied to the tissue for 1-min periods at intervals of 15 min. ^{3}H-GABA (0.05 µM) uptake by rat cortical slices (0.1×0.1×2 mm) and individual superior cervical ganglia and retinae was determined by the methods described previously[9–12]. Isoguvacine hydrochloride and N-methyl isoguvacine hydrochloride were prepared by the method of Oediger and Joop[13]. Their structures were confirmed by elemental analysis and carbon and proton magnetic resonance spectra. The samples of isonipecotic acid and muscimol were kindly given by Dr C. Cooksey, Department of Chemistry, University College London and Dr T. Inch, Chemical Defence Establishment, Porton Down, Salisbury respectively.

Results and discussion. All 4 analogues, muscimol, isoguvacine, N-methyl isoguvacine and isonipecotic acid depolarized the rat superior cervical ganglion in a manner consistent with an action at GABA receptors. They produced a rapid short-lasting depolarization without any subsequent hyperpolarization (cf. the action of the cholinomimetic carbachol in this tissue figure 2 and see also Brown et al.[14]). Responses to each analogue could be readily blocked by the GABA antagonists bicuculline methochloride (BMC) and isopropyl bicyclophosphate (IPTBO) as illustrated in the experiment shown in figure 2. Submaximal matched responses were obtained to GABA (10 µM), muscimol (1.8 µM), isoguvacine (62 µM), N-methyl isoguvacine (5.7 mM), isonipecotic acid (240 µM) and carbachol 14.0 µM). The addition of BMC (20 µM) completely blocked the responses to the analogues without suppressing that to carbachol. Recovery of the responses to all of the analogues was obtained 20 min after removal of the BMC. Subsequent superfusion with IPTBO (24 µM) also antagonized the responses to the analogues without affecting the responses to carbachol. Recovery was similarly obtained 20 min after superfusion with IPTBO-free solution. The log

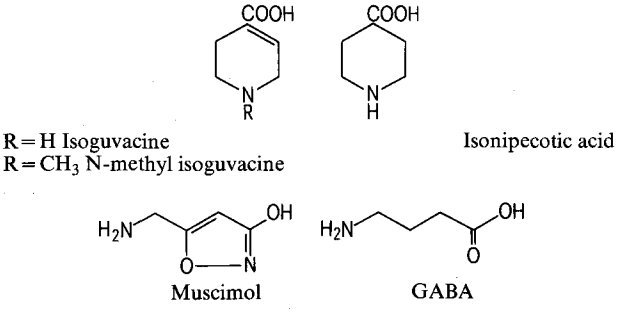

Fig. 1. Analogue structures.

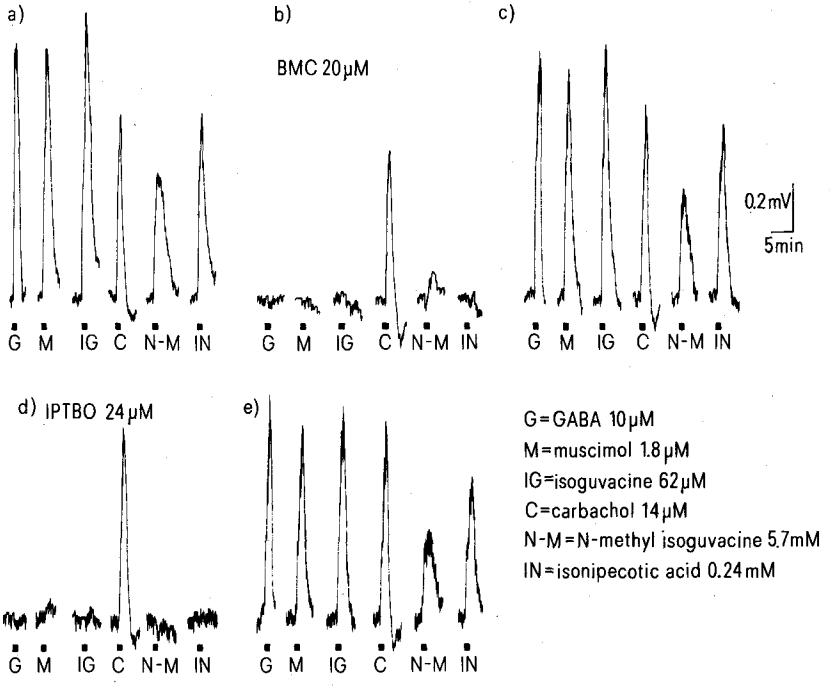

Fig. 2. Antagonism of the responses to the analogues produced by bicuculline methochloride (BMC) and isopropyl bicyclophosphate (IPTBO). Submaximal responses were obtained consecutively to GABA (G 10 µM), muscimol (M 1.8 µM), isoguvacine (IG 62 µM), carbachol (C 14 µM), N-methyl isoguvacine (NM 5.7 mM) and isonipecotic acid (IN, 240 µM). Each substance was applied for 1 min at intervals of at least 15 min indicated by the gaps between responses. Panel *a* control responses; *b* during continuous superfusion with BMC 20 µM; *c* recovery; *d* during IPTBO 24 µM; *e* recovery. Hyoscine hydrobromide (2.6 µM) was present throughout to restrict the action of carbachol to nicotinic receptors (see Bowery and Brown[6]). All the responses were obtained in a single ganglion. Calibration 0.2 mV and 5 min.

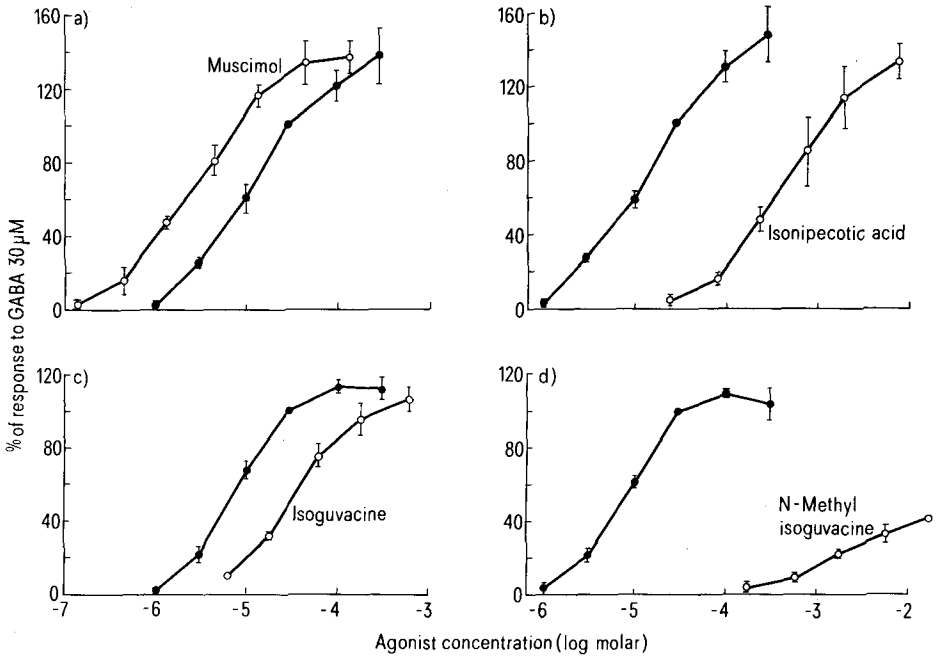

Fig. 3. Log dose/response curves for *a* muscimol; *b* isonipecotic acid; *c* isoguvacine; *d* N-methyl isoguvacine and GABA (●)-induced depolarization of the rat isolated superior cervical ganglion. Data were obtained from 3 (b and d) or 4 ganglia (a and c) for each compound. In each experiment comparison was made with GABA and responses were normalized with respect to that produced by 30 µM GABA to facilitate comparison between experiments. Each point is the mean value (vertical bars = SEM). Abscissa: log molar agonist concentration.

dose-response curves for muscimol, isoguvacine and isonipecotic acid were parallel to that for GABA (figure 3). Muscimol was the only analogue more potent than GABA (5.08±0.707 mean±SEM 4 experiments). The potency of isoguvacine was 0.23±0.026 (mean±SEM 4 experiments), and isonipecotic acid 0.011±0.0028 (mean ±SEM 3 experiments). N-methyl isoguvacine was a very weak agonist (< 0.001 GABA, figure 3).

Thus although the potency of muscimol in ganglia compares favourably with the values determined from iontophoretic and binding studies in central nervous tissue[3-5] the potencies of isoguvacine and isonipecotic acid appear to be at least an order of magnitude weaker in the ganglion than when applied iontophoretically to spinal interneurones[2].

It is unlikely that the weaker activity of these analogues can be attributed to any difference in their interaction with GABA transport processes in the central nervous system and ganglia (cf. the action of cis ±3-aminocyclohexanecarboxylic acid[15]) since we have not only confirmed the finding of others[2,16] that isoguvacine and isonipecotic acid have little or no effect on neuronal ^{3}H-GABA uptake in rat cortical slices but that they are also without effect on the glial ^{3}H-GABA uptake processes in sympathetic ganglia and rat retinae at concentrations up to 5 mM. In the presence of this concentration (5 mM) of isoguvacine and isonipecotic acid ^{3}H-GABA uptake into cortical slices was respectively 54.9±1.4% and 51.2±0.41% of control (mean±SEM n = 6 and 9. 10 min incubation at 25 °C) whereas the uptake values similarly obtained in sympathetic ganglia were 76.3±12.4% and 114.0±6.4% (n = 3 in each case. 30 min incubation) and in retinae 98.6±6.6% and 107.2±9.0% (n = 5 and 7. 10 min incubation) of controls respectively.

The weaker receptor activity could, of course, be due to a receptor difference between ganglia and central neurones. However, the potencies of isoguvacine and isonipecotic acid at central receptors have so far only been determined iontophoretically where concentrations are unknown. Their potencies relative to GABA were just assessed from currents required to produce equal and submaximal inhibitions of cell firing[2]. It will be interesting therefore to compare our results in ganglia with their relative potencies as inhibitors of ^{3}H-GABA binding where concentrations are known.

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