MODIFICATION BY DRUGS USED IN ANAESTHESIA OF CNS STIMULATION INDUCED IN MICE BY LAUDANOSINE AND STRYCHNINE†

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SUMMARY
We have investigated in mice the effects of several drugs which may be administered as part of an anaesthetic technique on the convulsive threshold to laudanosine and to strychnine, which is reported to have a similar mechanism of action. I.v. administered propofol, thiopentone and midazolam increased the dose of convulsant necessary to produce seizure when administered 2 min before the convulsive stimulus. In contrast, methohexitone and etomidate exhibited a proconvulsant effect, although with the latter this was significant only in laudanosine-treated mice. Pethidine was proconvulsant in both laudanosine- and strychnine-treated mice, but morphine was proconvulsant only in strychnine-treated mice. The effects of morphine, but not pethidine, were antagonized by naloxone 1 mg kg⁻¹. Laudanosine, but not strychnine caused arousal from anaesthesia in subconvulsive doses. This and other evidence suggests that the mechanism of the CNS excitation produced by strychnine and laudanosine are not the same.

KEY WORDS

Laudanosine is one of the major breakdown products of the neuromuscular blocking agent atracurium [1] and has been shown to have potent convulsive action in dogs [2] and rats [3]. Many agents used during anaesthesia have both pro- and anti-convulsant activity. For example, barbiturates such as phenobarbitone may be anti-convulsant at doses causing little sedation, and others such as methyl-thiobutobarbitone have so many excitatory effects such as gross limb movement that their use as anaesthetic agents is not feasible [4]. The clinically useful i.v. anaesthetics have activity profiles between these two extremes. The isomer of methohexitone marketed as an i.v. anaesthetic is the alpha-DL isomer, as the beta isomer had too much intrinsic excitatory activity to be clinically useful. Even so, methohexitone as marketed has been reported to have more proconvulsant activity than thiopentone. Indeed, methohexitone has been used to provoke epileptiform activity in patients with seizure patterns that were not revealed by awake records [5]. In contrast, thiopentone is reported not to provoke an epileptic response in such patients [6]. Nevertheless, many authors do not feel that methohexitone is contraindicated in patients with epilepsy [7–9] and its use to control seizures has been advocated by some [4]. Similar controversy exists concerning the pro- and anti-convulsant profile of other drugs used in anaesthesia. Midazolam, with pronounced anti-convulsant properties [10], has been reported to produce seizures in several instances [11]. Propofol has been reported to reduce the duration of ECT-induced seizures [12–14] and to be a useful agent in status epilepticus [15]. Despite these reports, several case reports have appeared in the literature reporting convulsions, opisthotonus or epileptiform EEG activity in patients given...
propofol [16, 17]. In addition, opioid drugs and endogenous peptides have been implicated in pro- and anticonvulsant activity [18].

Avramov and Mori [19] have demonstrated that nitrous oxide antagonizes the CNS stimulating action of laudanosine and this raises the question as to how other drugs used in anaesthesia might modify the excitatory action of laudanosine. The present study was designed to investigate this question in the experimental animal. A range of i.v. induction agents have been used, together with midazolam and the opioid drugs morphine and pethidine. Morphine was selected as the standard opioid drug with which others are compared and pethidine was chosen as it is particularly prone to excitatory side effects [20].

The mechanism of the excitatory action of laudanosine is uncertain, but is thought to be related to antagonism of glycine receptors in a manner similar to that for strychnine [21]. This was supported by the later work of Pong and Graham [22] using rat electoretinography as a screen for glycine antagonists. Since Soar and colleagues [23] have implicated glycine antagonism in the excitatory actions of some i.v. anaesthetic agents, it was decided to compare the interactions of laudanosine and drugs used in anaesthesia with those of the standard glycine antagonist, strychnine.

MATERIALS AND METHODS

Female MF1 mice weighing 25–35 g were used in the study, which was approved by the Home Office under the Animals (Scientific Procedures) Act 1986 (licence number 50/00609). All experiments were carried out between 10:00 and 14:00. Each mouse was weighed and injected with opioid drug, i.v. anaesthetic agent or vehicle. The opioids were injected i.p. (0.1 ml per 10 g body weight) 15 min before testing and the i.v. drugs were injected (0.1 ml per 10 g body weight) into the lateral tail vein over a 10-s period 2 min before testing. I.v. injections were carried out using a winged-needle infusion set (Butterfly-27, Abbott Laboratories Ltd) with an outside diameter of 0.4 mm and length of 9.5 mm attached to a 1-ml syringe. All i.v. injections took place in a specially designed box which allowed movement of the mouse with the exception of the tail, which was gently held. The minimum convulsant dose for laudanosine and strychnine was obtained by infusing the convulsant agent at a rate of 0.12 ml min⁻¹ into a second tail vein until a full seizure occurred, at which point the animal was killed immediately by an overdose of anaesthetic before consciousness returned. The dose was calculated as the total dose of convulsant infused divided by body weight. The dose that induced arousal from anaesthesia was noted also in the relevant experiments. In all experiments, the results from drug-treated mice were compared with those for vehicle-treated mice which were tested concurrently using randomly selected cage mates. A single observer was responsible for all animals tested in a given drug group—for example all morphine experiments or all thiopentone experiments. Doses of drugs were selected which had significant pharmacological activity in the mouse. Usually, these data were obtained from previous studies by this anaesthetic research group and are compatible with the literature. In preliminary experiments at least two doses of each i.v. anaesthetic were selected: a small dose which anaesthetized all mice within 10 s and induced sleeping times of 3–5 min, and a larger dose which induced 7–10 min sleep with no fatalities. Insufficient data were produced in these dose finding studies to analyse statistically the sleeping time results.

Laudanosine (Sigma Chemical Co.) and strychnine (BDH) were dissolved in 0.9 % saline using an ultrasonic shaker and a few drops of hydrochloric acid 1 mol litre⁻¹ and adjusted to pH 6.8 with sodium bicarbonate. The final concentrations for infusion were 2 mg ml⁻¹ for laudanosine and 100 μg ml⁻¹ for strychnine.

Thiopentone (Intraval Sodium, May & Baker Pharmaceuticals) and methohexitone (Brietal, Eli Lilly & Co., Ltd) were prepared freshly every day from the commercially available ampoules by dissolving the dry powder in saline. Propofol (Diprivan, ICI Pharmaceuticals) and etomidate (Hypnormidate, Janssen Pharmaceuticals Ltd) were diluted with normal saline immediately before injection, dilutions of Intralipid (Kabi Vitrum) or propylene glycol (BDH) being used as the vehicle control. Morphine hydrochloride (Macfarlane Smith Ltd) and midazolam (Roche Products Ltd) were prepared by dissolving the dry powder in saline, whilst pethidine was diluted with normal saline from pethidine injection 50 mg ml⁻¹ (MacCarthys Ltd). Although results are described as mean values (SEM) (unless otherwise stated, n = 10) for ease of presentation, statistical comparisons were performed using the
Mann–Whitney U test to avoid assumptions on the distribution of data.

RESULTS
In saline-treated control animals, laudanosine i.v. caused no visible behavioural changes until seizures occurred with a dose of 16.1 (0.5) mg kg\(^{-1}\). The seizure consisted of an asymmetrical clonic convulsion which proceeded into a tonic phase if the animal was not killed immediately after the appearance of the convulsion. In contrast, strychnine induced a seizure characterized by symmetrical tonic extension of the body and limbs with no evidence of a clonic phase; the dose necessary was 0.61 (0.02) mg kg\(^{-1}\). In the presence of the anaesthetic agents, laudanosine, but not strychnine, caused arousal before the onset of the convulsion. Arousal in the mice given the larger doses of anaesthetic agent occurred when about 50% of the convulsant dose had been injected (table I). (In the lower dose experiments arousal and natural waking could be confused.)

Thiopentone induced anaesthesia in mice with no abnormal movements of the limbs. Two minutes after the administration of thiopentone, the dose of laudanosine necessary to induce convulsions was significantly increased (table II). Additional experiments showed that this protective effect outlasted the anaesthetic action of the drug as, 15 min after injection of thiopentone 40 mg kg\(^{-1}\), the convulsive dose of laudanosine had increased further to 25.1 (1.3) mg kg\(^{-1}\). Thiopentone in doses up to 40 mg kg\(^{-1}\) had no protective effect against strychnine-induced seizures.

In contrast, methohexitone reduced the doses of both strychnine and laudanosine necessary to
induce convulsions, this being significant with the 20 mg kg\(^{-1}\) dose (table II). However, this dose of methohexitone produced anaesthesia with visible cyanosis. Smaller doses were not proconvulsant and, indeed, the 10-mg kg\(^{-1}\) dose significantly protected mice against laudanosine-induced seizures, even though the mice exhibited myoclonic jerks and opisthotonus in response to the anaesthetic agent alone. A similar pattern of responses was seen with etomidate and laudanosine (table II), in that 6.7 mg kg\(^{-1}\) had a proconvulsant effect, whilst the smaller dose was protective against laudanosine-induced convulsions but caused twitching and myoclonic jerks when administered alone. An intermediate dose of etomidate (5 mg kg\(^{-1}\)) was tested to determine the dose at which the changeover from anticonvulsant to proconvulsant effects occurred. No opisthotonus occurred with etomidate. In contrast with methohexitone, the proconvulsant dose of etomidate in laudanosine-infused mice had no significant effects in strychnine-infused mice.

Propofol produced a dose-dependent anticonvulsant effect against both compounds (table II). An additional dose of propofol, which induced an unsteady gait but no loss of righting reflex, still produced an anticonvulsant effect. The largest dose caused anaesthesia with visible cyanosis. No abnormal limb movements were seen when propofol was administered i.v.

Midazolam also was anticonvulsant in both seizure models (table II), although it clearly provided greater protection against laudanosine than strychnine.

Morphine 2.5-20 mg kg\(^{-1}\) had no effect on laudanosine-induced convulsions, but produced a dose-dependent proconvulsant effect in mice infused with strychnine. This effect was abolished by the administration of naloxone 1 mg kg\(^{-1}\) (fig. 1). Pethidine was proconvulsant in both seizure models—an action which was unaffected by administration of naloxone (fig. 2).

**DISCUSSION**

Many anticonvulsant drugs increase gamma-aminobutyric acid (GABA) concentrations or enhance inhibitory transmission through GABA\(_A\) receptors [24] and this may explain the anticonvulsant actions demonstrated for many agents in this study. Propofol at anaesthetically relevant concentrations enhances GABA-mediated transmission [25], as do other anaesthetic agents including the barbiturates, etomidate and the benzodiazepines [26].

The two i.v. anaesthetic agents associated most often with excitatory phenomena, etomidate and methohexitone, have been shown to possess significant proconvulsant activity. However, this proconvulsant activity was not seen with small...
doses of these agents which produce the most obvious excitatory effects.

Soar and colleagues [23] proposed that methohexitone and propofol might exert excitatory effects by virtue of glycine antagonism, as their excitatory effects were potentiated by low doses of the glycine antagonist, strychnine, but not by low doses of bicuculline, an antagonist at GABA<sub>α</sub> receptors. Whilst the present work with methohexitone is compatible with this conclusion, no proconvulsant effects were detected for propofol, even when strychnine was the convulsive stimulus. Indeed, propofol was convincingly anticonvulsant in this seizure model. However, there are many methodological differences between the two series of experiments, the most important probably being the route of injection. The present study used exclusively the i.v. route, whilst Soar and colleagues’ [23] experiments used the i.p. route. With the latter, much greater whole body doses are required to produce anaesthesia, the concentrations in the biophase remain increased for longer and a greater degree of metabolism will have occurred. In addition, when a drug is given i.p., the progression towards anaesthesia is slower and an excitatory action may be revealed which would have been swamped by an anticonvulsant action if the drug was given i.v. An anticonvulsant action of i.v. propofol against strychnine-induced convulsions has been demonstrated by Hogskilde [unpublished data on file, ICI Pharmaceuticals]. In patients with epilepsy, etomidate, etomidate has been reported to have proconvulsive activity [27–29], [31]. In the present study, it was possible to demonstrate a proconvulsant action for etomidate only when laudanosine was the convulsive stimulus; even then, the effect obtained was dependent upon the dose used. Etomidate had no effect on strychnine-induced seizures and was anticonvulsant in pentylenetetrazole-induced seizures [31]. It is clear that laudanosine- and strychnine-induced convulsions differ both in their gross characteristics and in the way they are modified by drugs used in anaesthesia. This indicates that there is not a common mechanism for the seizures produced by the two convulsant agents. Neither opioid tested exhibited any anticonvulsant activity: pethidine was proconvulsant in both seizure models, whilst morphine was proconvulsant only in the strychnine model. The proconvulsant action of morphine in strychnine-treated animals was naloxone sensitive and probably involves classical opioid receptors. A similar interaction between morphine and strychnine has been reported in other species [18].

Pethidine and morphine have a similar selectivity for the subtypes of opioid receptors [32]. However, pethidine or, more likely, its metabolite norpethidine has been shown to be a convulsant in man and animals and this effect is potentiated by naloxone [20]. Thus it is not surprising that the proconvulsant activity of pethidine described in the present work was not naloxone sensitive. It is probable also that pethidine is not exerting its effects through opioid receptors. Pethidine-induced hypothermia in the mouse is also insensitive to naloxone and an involvement of tryptaminergic mechanisms has been proposed [33]. The involvement of tryptaminergic mechanisms in the proconvulsant action of pethidine has yet to be investigated.

REFERENCES
13. Dwyer R, McCaughrey W, Lavery J, McCarthy G, Dundee JW. Comparison of propofol and methohexitone...


