

Inducing Anesthesia with a GABA Analog, THIP

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The authors have postulated previously that general anesthetic agents act via a potentiation of the inhibitory action of gamma-aminobutyric acid (GABA) at central synapses. If the hypothesis is true, GABA should induce anesthesia, however, GABA itself does not pass through the blood-brain barrier. A GABA analog was sought as a substitute to test the authors' hypothesis. A new bicyclic GABA analog, THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol) was selected because its properties are similar to GABA *in vitro*. THIP was found to induce anesthesia in rodents, and its behavior was compared with that of thiopental, ketamine, midazolam, and gamma-hydroxybutyrate. Complete loss of righting reflex occurred with doses of THIP and thiopental just under 100 $\mu\text{mol/kg}$, with ketamine and midazolam less than 50 $\mu\text{mol/kg}$ and with gamma-hydroxybutyrate of more than 6,000 $\mu\text{mol/kg}$. Complete recovery from thiopental and ketamine occurred in less than 5 min, with midazolam recovery required about half an hour and with gamma-hydroxybutyrate and THIP it took about 1½ h. THIP induced analgesia as well as sedation and loss of righting reflex. Recovery was complete, and no adverse effects were noted in these rodents. (Key words: Anesthetics, intravenous: THIP. Brain: GABA receptors. Neurotransmitters: GABA. Receptors: GABA.)

GAMMA-AMINO BUTYRIC ACID (GABA) is an established central inhibitory neurotransmitter, and we have proposed that general anesthetic agents manifest their pharmacologic actions via a potentiation of this inhibitory action of GABA on synaptic transmission. This hypothesis is based on a correlation of anesthetic potency of each agent to its inhibitory action on GABA disposal in a synaptosomal model.¹ Receptor binding experiments^{2,3} and neurophysiologic studies^{4,5} support this hypothesis. The hypothesis cannot be tested directly by systemic administration of GABA because it does not cross the blood-brain barrier.

We have sought a GABA analog (fig. 1) that could cross the blood-brain barrier and mimic GABA action *in vivo*. Gamma-hydroxybutyric acid (GHB) and its lactone cause anesthesia, but massive amounts must be employed.⁶ A limitation on conversion from GHB to GABA could explain this observation.⁷ Of the newer analogs, isoguavacine, muscimol, and THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol) were all found to mimic GABA in *in vitro* studies and THIP was proposed to be the most promising GABA substitute.⁸

THIP is a GABA analog with a complex cyclic structure (fig. 1). Electrophysiologically, it acts like GABA

toward bicuculline inhibition of spinal interneurons.⁹ Although it binds to GABA receptors with lesser affinity than GABA,¹⁰ it still remains as one of the few GABA analogs that shows high affinity for those receptors. It protects against seizures in mice but not in baboons,¹¹ and it causes analgesia in mice.¹²⁻¹⁴ THIP also exerts certain pharmacologic effects in humans.¹⁵⁻¹⁸ It reduces the monosynaptic T-reflex and reinforces vibratory inhibition of the I_A monosynaptic pathway.¹⁵ It has no effect on seizures at maximum doses, but a trend is observed for lower seizure frequency during a period on submaximal dose.¹⁶ Its anxiolytic effects are weak,¹⁷ and its analgesic effects are observed at dose levels that also cause side effects.¹⁸ In clinical studies,¹⁵⁻¹⁸ the primary side effects are sedation and dizziness. No effect is found on blood pressure, respiration, heart rate, blood and urine tests. We tested THIP for its anesthetic effects in rats and mice and compared it with several intravenous anesthetic agents.

Methods

Timed observations of anesthetic effects were made in rats (table 1). Concurrent observations of analgesia were made in the same rats with the use of the hot plate escape and tail pinch response tests.¹⁹ Separate assays of analgesic effects were performed with the use of the mouse radiant heat-tail flick test.²⁰ Timed observations of anesthetic effects were repeated in mice only for THIP (table 2).

Drugs dissolved in saline were infused through the tail vein of young albino male Sprague-Dawley rats (125-200 g; BioLab, Oak Park, Illinois) via a 25-gauge butterfly needle and followed by 0.5 ml of saline. Drugs studied and doses administered are listed in table 1. They were obtained from the following sources: Abbott (thiopental), Park-Davis (ketamine), Roche (midazolam), and Sigma (GHB). THIP was kindly supplied to us by Dr. Krosggaard-Larsen and by H. Lundbeck & Co. of Denmark. Albino male Swiss-Wistar mice (15-25 g; BioLab), injected intraperitoneally with all drugs except thiopental, were used in the radiant heat-tail flick analgesia assay.

Intervals between drug administration and the onset of specific behavioral changes were timed by stopwatch with zero time being the completion of the 0.5-ml saline flush. A reproducible behavioral sequence occurred as follows: 1) L-ex—loss of spontaneous exploration. 2) S-rr—slowing of respiratory rate. Sometimes the animal gasped or hiccuped. 3) L-rr-1—some loss of righting

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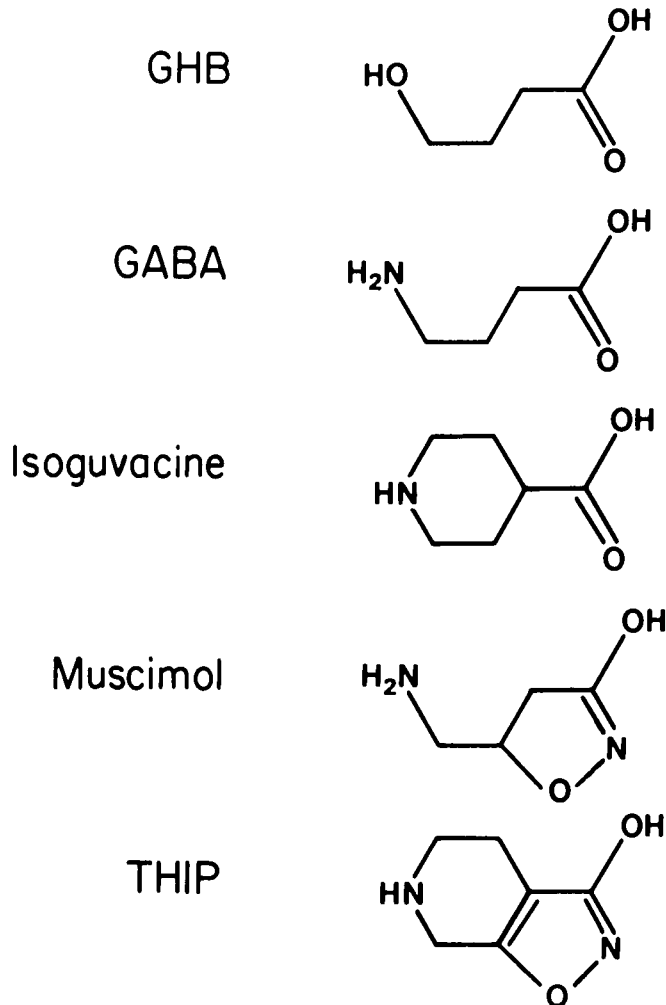


FIG. 1. Structural formulae of some pharmacologically active GABA analogs.

reflexes with spreading of hind limbs in a broad base stance. At this stage, animals were placed in the lateral recumbent position every 15 s and observed for further response. (Normal animals immediately assumed the upright position.) 4) L-rr-2—return of head and front paws to upright position but inability to return hind limbs from lateral recumbent position. 5) L-rr-3—all righting abilities lost. Animals remain on their backs, often with their eyes closed. 6) R-rr—spontaneous resumption of the upright position, *i.e.*, return of the righting reflexes. Usually the animals remain at the same spot for some more time. The return of righting reflexes did not involve a slow progression through several clearly defined stages, as did the loss of righting reflexes, but rather occurred as a specific event. 7) R-ex—sudden recovery of spontaneous exploration.

Concurrent observations of analgesic effects were made in anesthetized rats using the 56° C hot plate escape (15 s) and the tail pinch response (movement and/or vocalization) tests.¹⁹ These observations were made at all doses of all drugs listed in table 1.

In addition, analgesia in mice was tested separately with the use of the mouse radiant heat-tail flick test.²⁰ Drugs were injected intraperitoneally at the following doses ($\mu\text{mol}/\text{kg}$): ketamine, 42, 84, and 126; midazolam, 100; GHB, 4,000; and THIP, 45 and 90.

Means and standard errors, with Student's correction for small sample sizes,²¹ of the timed durations ($N = 6$) are given in "Results."

Results

Onset and recovery intervals with the five drugs studied in rats are reported in table 1. The effects of

TABLE 1. Anesthetic Observations in Rats with Several Intravenous Anesthetics

Drug ($\mu\text{mol}/\text{kg}$)	Thiopental 97	Ketamine			Midazolam		GHB		THIP			
		38	51	63	50	81	6000	8000	68	90	113	135
L-ex	F	F	F	F	0.1 <i>0.1</i>	0.2 <i>0.1</i>	2.0 <i>0.3</i>	1.0 <i>0.4</i>	—	1.1 <i>0.6</i>	0.3 <i>0.1</i>	0.1 <i>0.1</i>
L-rr-1	F	F	F	F	F	0.2 <i>0.1</i>	2.9 <i>0.5</i>	1.1 <i>0.5</i>	—	0.3 <i>0.2</i>	0.2 <i>0.1</i>	0.2 <i>0.2</i>
S-rr	F	F	F	F	0.4 <i>0.3</i>	0.4 <i>0.1</i>	6.8 <i>1.4</i>	4.1 <i>1.9</i>	—	2.6 <i>0.4</i>	3.0 <i>0.9</i>	2.4 <i>0.8</i>
L-rr-2	F	F	F	F	0.6 <i>0.3</i>	0.3 <i>0.1</i>	8.9 <i>1.6</i>	5.2 <i>1.2</i>	NQ	4.1 <i>1.0</i>	2.3 <i>0.9</i>	0.6 <i>0.4</i>
L-rr-3	F	F	F	F	1.5 <i>0.8</i>	0.5 <i>0.1</i>	NQ	7.5 <i>1.4</i>	NQ	NQ	8.7 <i>2.2</i>	4.9 <i>1.7</i>
R-rr	5.1 <i>1.4</i>	2.1 <i>0.3</i>	2.4 <i>0.2</i>	4.6 <i>0.4</i>	11 <i>3</i>	25 <i>4</i>	45 <i>7</i>	40 <i>4</i>	NQ	65 <i>13</i>	85 <i>13</i>	88 <i>13</i>
R-ex	—	2.7 <i>0.4</i>	3.1 <i>0.3</i>	5.3 <i>0.4</i>	34 <i>12</i>	42 <i>16</i>	85 <i>5</i>	122 <i>19</i>	91 <i>34</i>	113 <i>3</i>	137 <i>5</i>	154 <i>4</i>

Values in minutes to attain various anesthetic states and recovery; means \pm standard errors (*italics*) with $N = 6$; F = too fast to measure;

— = no record; NQ = never reached this stage. Data under 68 $\mu\text{mol}/\text{kg}$ of THIP was based on three rats.

THIP in mice are reported in table 2. Both thiopental and ketamine caused rapid induction and recovery from anesthesia in rats as judged by our criteria. The onset of midazolam anesthesia was rapid, but recovery was slow. GHB caused a slower induction and recovery. THIP effects were much slower than those of thiopental and ketamine but slightly faster than GHB. THIP produced a much more prolonged anesthetic state than all other drugs tested. A positive correlation was established between rapidity of onset and duration of action with THIP in both rats and mice (tables 1 and 2; figs. 2 and 3). The molar concentration of THIP required was in the same range as thiopental but slightly greater than with ketamine and midazolam (table 1). The required GHB dosage was two orders of magnitude larger.

With onset of effect with all drugs studied, respiration slowed and sometimes became periodic in nature. Occasionally, especially in mice, hiccuping occurred for a short period. Most rats closed their eyes after loss of righting reflex, but this usually was not observed in the mice. With THIP, rats tended to spin their tails in a conical pattern at some time during anesthesia. Recovery from anesthesia through the three stages of righting reflex was difficult to quantitate. On recovery from THIP, GHB, and midazolam, the rodents righted themselves directly into a broad base stance but did not resume exploratory behavior for variable periods of time (from 20–70 min), depending on drug and dosage. Higher doses of THIP and GHB were associated with prolonged recovery of exploratory behavior. Recovery of righting reflex and of exploration was most delayed with THIP. With ketamine, the rats started to explore

TABLE 2. Anesthetic Observations in Mice with THIP (values in min)

$\mu\text{mol/kg}$	63	126	189	253
L-ex	4.3 <i>1.4</i>	2.8 <i>0.4</i>	1.9 <i>0.1</i>	1.8 <i>0.3</i>
L-rr-1	6.5 <i>2.0</i>	3.3 <i>0.6</i>	2.5 <i>0.3</i>	2.3 <i>0.3</i>
S-rr	6.8 <i>2.8</i>	4.9 <i>0.5</i>	3.8 <i>0.2</i>	3.6 <i>1.1</i>
L-rr-2	NQ	8.2 <i>1.3</i>	4.4 <i>0.3</i>	4.0 <i>0.6</i>
L-rr-3	NQ	8.8 <i>1.5</i>	5.9 <i>0.5</i>	4.5 <i>0.5</i>
R-rr	NQ	46 <i>15</i>	83 <i>6</i>	97 <i>19</i>
R-ex	91 <i>29</i>	109 <i>7</i>	112 <i>10</i>	152 <i>22</i>

Values in minutes to attain various anesthetic states and recovery; means \pm standard errors (*italics*) with N = 6; F = too fast to measure; — = no record; NQ = never reached this stage. Data shown under dosage 63 $\mu\text{mol/kg}$ body weight was based on only three mice.

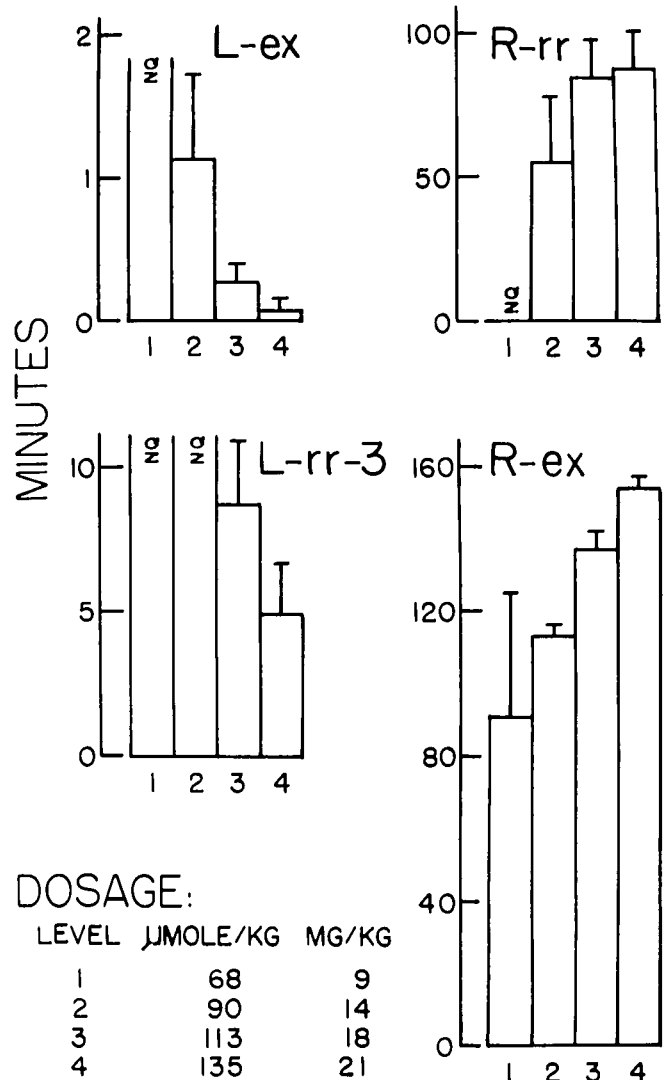


FIG. 2. Anesthetic effects of THIP in rats. Abscissa—dosages; ordinates—time in minutes to reach increasing depth of anesthesia or recovery; L-ex—loss of exploration; L-rr-3—complete loss of righting reflex; R-rr—regain of righting reflex; and R-ex—resumption of exploration. N = 6; NQ = never reached this stage.

within a minute of recovery, but locomotion was unstable for about another 5 min. All animals tested with THIP and with anesthetic doses of other drugs recovered fully.

As measured by both the hot plate escape and tail pinch withdrawal tests,¹⁹ THIP did not cause analgesia in rats at any dosage. GHB in rats was analgesic with tail pinch but not with the hot plate test. No analgesia was observed with subanesthetic doses of thiopental, ketamine, or midazolam with the use of these tests in rats. In mice, with the use of the radiant heat–tail flick test,²⁰ ketamine, midazolam, GHB, and THIP all caused analgesia. The onset and the duration of the analgesic effects of these drugs were shortest with ketamine and longest with THIP.

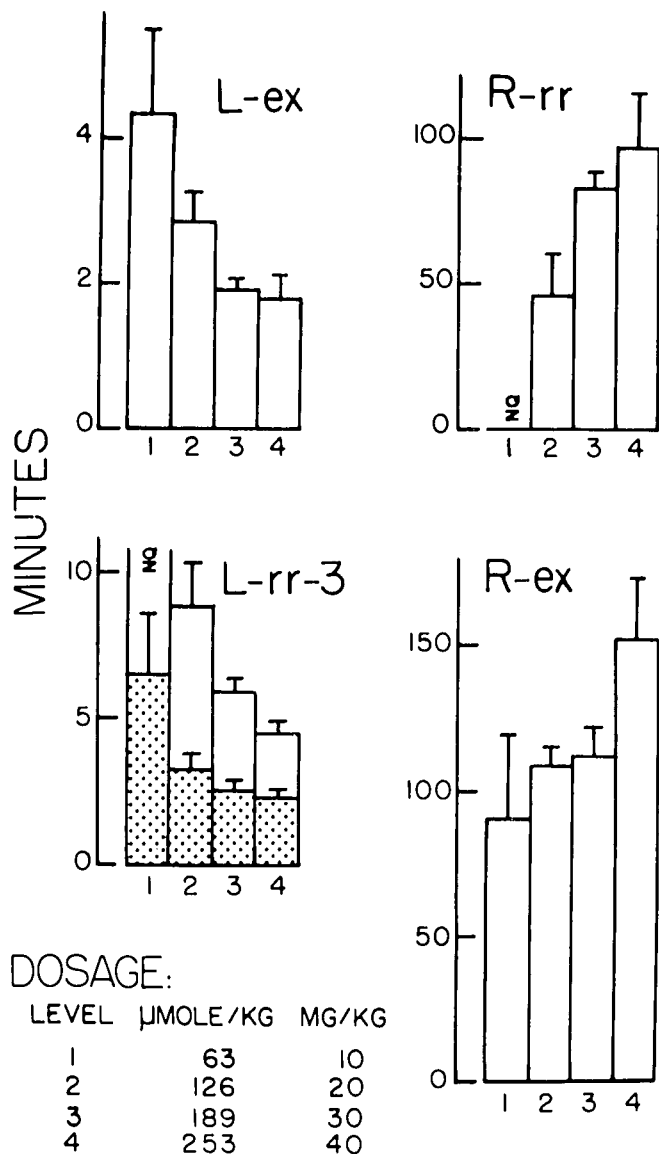


FIG. 3. Anesthetic effects of THIP in mice. Abscissa—dosages; ordinates—time in minutes to reach increasing depth of anesthesia or recovery; L-ex—loss of exploration; L-rr-3—complete loss of righting reflex; R-rr—regain of righting reflex; R-ex—resumption of exploration. L-rr-1—onset of broad base stance is represented by the dotted area in L-rr-3.

Discussion

THIP induced an anesthesia-like state in rats at molar concentrations in the same range as thiopental. Dose requirements of THIP were slightly higher than those for ketamine but much lower than those for GHB. Onset of THIP action was dose dependent and significantly slower than with thiopental or ketamine but faster than with GHB. Time of onset of THIP anesthesia was similar to that of midazolam. Duration of THIP action exceeded that of any other drug in this study. These observations suggest that THIP may have potential for clinical usage. Indeed, sedation was the most commonly

observed side effects in clinical trials with THIP for seizure and anxiety relief and for analgesic and neurologic effects.¹⁵⁻¹⁸

In assessing analgesia, we found difficulty with each of the three methods employed. In the hot plate and tail pinch tests, the response was difficult to assess with heavy sedation. Furthermore, the stimulus strengths administered in the tail pinch test could not be quantitated. The radiant heat test, which was well suited for mice, was rather insensitive in rats, probably because of heavy tail cornification. The analgesic efficacy of these drugs therefore was tested more thoroughly in the mice, although observations were made in the rats at the time anesthetic effects were observed. Onset and duration of analgesia in mice was shortest with ketamine and longest with THIP, a pattern similar to their anesthetic effects in rats. Pain relief in cancer patients using THIP was unsuccessful mainly because the doses required for analgesia also caused sedation and dizziness.¹⁸ The analgesic effect of THIP was postulated to be mediated via a different mechanism than that with opiates.^{12-14,22-24}

Respiratory depression was a prominent feature of THIP action, as it was with all of the other anesthetics agents studied. Hiccups of brief duration, reminiscent of methohexital hiccups, occasionally was observed. This was not noted during clinical trials.¹⁵ No other toxic effects were seen. All animals recovered spontaneously, and no delayed mortality was observed.

The effect of THIP in rats and mice appeared to start from the lower spinal cord and proceed upward toward the brain. Invariably, the righting reflex in the hind legs was lost before the front legs were affected. Next, the righting reflex was completely lost but eyes remained open, although the animal was not responsive. Eventually, most rats closed their eyes and appeared to sleep. These observations, independent of the route of drug administration, suggest that the spinal neurons may be more sensitive to THIP than the central neurons.

It is obvious from these and other observations^{25,26} that THIP is a potent central nervous system depressant that may have potential to be developed as an anticonvulsant, sedative, and/or general anesthetic agent. As an anesthetic, it appears to have a reasonable margin of safety in the animal species in which it has been administered.

We postulated previously that potentiation of GABA inhibition may constitute a basis for anesthesia.^{1,2,27} The present study has shown that a GABA analog, THIP, caused anesthesia at a molar dose level similar to that required by other clinically used injectable anesthetics.

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