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Blockade of the reticulospinal inhibitory pathway by nitrous oxide and tetrodotoxin

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Summary

1. The effects of nitrous oxide and tetrodotoxin were investigated on the reticulospinal inhibitory pathway in the acutely decerebrated cat.

2. Both agents abolished the reticular inhibition of the segmental reflex potentials before they blocked either the segmental reflex potentials or the reticular facilitation of the reflex potentials.

3. These results confirmed the hypothesis that preferential block of the reticulospinal inhibitory pathway might be an important neural mechanism of the excitement stage of anaesthesia.

Introduction

The excitement stage of anaesthesia has been assumed to be due to the preferential block of inhibitory pathways by lower doses of anaesthetic agents. Evidence supporting this type of mechanism was reported by Preston & Whitlock (1960) who observed much higher susceptibility of the precentral inhibition of spinal reflexes to pentobarbital depression than the precentral facilitation. Similarly in recent work from our laboratory it was found that pentobarbital, paraldehyde, tribromethanol, chloralose and procaine completely blocked the reticulospinal inhibitory pathway before they blocked the related excitatory pathways (Frank & Ohta, 1971). The hypothesis was proposed that the preferential block of this inhibitory pathway might be an important neural mechanism of the excitement stage of general anaesthesia. This hypothesis was confirmed using nitrous oxide in the present investigation.

It is well known that these anaesthetic agents depress the initial increase in sodium conductivity as well as the secondary increase in the potassium conductivity which normally follows an adequate stimulation in nerves and skeletal muscles (Thesleff, 1956; Shanes, Freygang, Grundfest & Amatniek, 1959; Taylor, 1959; Inoue & Frank, 1962; Frank & Sanders, 1963). The blockade of the increase in sodium conductance was thought to be more important for anaesthetic depression than the reduction of the increase in potassium conductance (Thesleff, 1956; Frank, 1968; Frank & Sanders, 1963; Frank & Pinsky, 1966; Inoue & Frank, 1962, 1965). On the other hand, Frank & Pinsky (1966) suggested the possibility that suppression of the increase in potassium conductance by the anaesthetics might be involved in the production of the excitement stage of the general anaesthesia since no signs of pure central nervous system excitement produced by tetro-

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dotoxin were observed in their experiments and this drug is known to inhibit selectively the increase in sodium conductance (Narahashi, Deguchi, Urakawa & Ohkubo, 1960; Narahashi, Moore & Scott, 1964). Therefore it was of great interest to test tetrodotoxin on the reticulospinal inhibitory pathway and this was done in the present study. The results obtained did not support the suggestion by Frank & Pinsky (1966).

An account of some of these results was given to the Canadian Federation of Biological Societies (Ohta & Frank, 1970).

Methods

Adult cats (2·0–3·5 kg) were decerebrated at the intercollicular level under ether anaesthesia which was discontinued just before decerebration. Recordings were begun more than 4 h after discontinuation of ether. Laminectomy was made and the ventral roots from L5 to L7 were cut to reduce the movements during dorsal root stimulation. Segmental reflex potentials were recorded from the L6 ventral root responding to electrical stimulation of the ipsilateral L6 dorsal root. The stimulus voltage was supramaximal for the segmental reflex potentials. The ipsilateral bulbar reticular formation (dorsomedial part) was electrically stimulated through a stereotaxically oriented bipolar platinum electrode with repetitive square wave pulses of 2·5 ms in duration and of about 5 V in amplitude at 100 Hz/s for 10 to 15 seconds. Before drug application, the reflex potentials were greatly inhibited during adequate stimulation of the reticular formation. The amplitudes of the reflex potentials inhibited or augmented during reticular stimulation were plotted on graphs as % of the control amplitudes without reticular stimulation. The size of the polysynaptic potential was measured by its maximum height. The position of electrode in the reticular formation was histologically confirmed in every case reported in the present paper (see Frank & Ohta (1971) for the stereotaxic coordinates which were the same as those used here).

Nitrous oxide mixed with oxygen was introduced through a tracheal cannula. Tetrodotoxin, supplied by Sankyo Drug Co., was administered intravenously.

Artificial respiration was used when the breathing of the animal was much reduced or abolished by tetrodotoxin. The rectal temperature was maintained at $36 \pm 1°$ C. There was no significant change in either the heart rate or the ECG unless the dose of tetrodotoxin was so high that reflex potentials were abolished.

Results

1. *Nitrous oxide* (Figs. 1 and 2)

Nitrous oxide depressed both reflex potentials gradually and progressively (Fig. 1). The monosynaptic potential was a little more sensitive to depression by nitrous oxide than the polysynaptic potentials as previously reported for pentobarbital and paraldehyde (Frank & Ohta, 1971). This confirmed the observation of DeJong, Robles, Corbin & Nace (1968) who found that the polysynaptic potentials were more resistant than monosynaptic potentials to block by some gaseous and volatile anaesthetics.

The reticular inhibition of the reflex potentials was gradually and progressively reduced by nitrous oxide up to concentrations of 40% and it was then rapidly

depressed and finally abolished by 50 to 80% nitrous oxide. The mean blocking dose was $62 \pm 3{\cdot}4\%$ nitrous oxide for the reticular inhibition of the monosynaptic potential and $64 \pm 4{\cdot}6\%$ for the reticular inhibition of the polysynaptic potentials. At the mean blocking doses, the monosynaptic potential was reduced to about 40% of the control (no drug) value and the polysynaptic potentials were reduced to about 60% of the initial amplitudes.

After the reticular inhibition was abolished, reticular stimulation at a higher intensity with the same electrodes augmented the reflex potentials as shown in the bottom row in Figure 2. This reticular facilitation of the reflex potentials was probably produced by the spread of current to the excitatory pathway in the vicinity of the inhibitory region. This reticular facilitation could not be abolished by a gas mixture containing 85% nitrous oxide and further doses were not tested because they caused a fatal oxygen deficiency.

2. *Tetrodotoxin* (Figs. 3 and 4)

Tetrodotoxin, almost up to a dose at which reticular inhibition was blocked, augmented the monosynaptic potential and further doses very rapidly depressed the monosynaptic potential as shown in Figure 3. The polysynaptic potentials were slightly depressed by relatively low doses (up to 6 μg/kg) and rapidly depressed

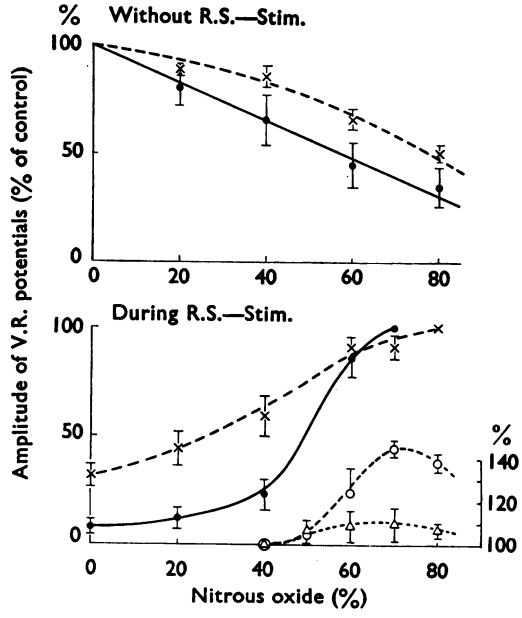

FIG. 1. The effect of nitrous oxide on the segmental reflex potentials is shown in the upper diagram. Each dot (monosynaptic) and cross (polysynaptic) indicates the mean of 5 observations. The lower diagram illustrates the effect of nitrous oxide on the reticular inhibition (curves at the left) and facilitation (curves at the right) of the reflex potentials. The dot and cross indicate each mean of the inhibited mono- and polysynaptic potentials respectively during reticular stimulation and 100% indicates a complete block of inhibition in every case. The open circle and the open triangle indicate the augmented mono- and polysynaptic potentials respectively during reticular stimulation with a higher intensity after abolition of inhibition. The calibration of these facilitated reflex potentials is shown at the right. Note that the baseline is 100%.

by higher doses of tetrodotoxin. The monosynaptic potential was more resistant to tetrodotoxin than the polysynaptic ones and this confirmed the earlier observation made by Kuriaki, Hiyoshi & Mochizuki (1956).

The reticular inhibition of both reflex potentials was progressively reduced and finally abolished by tetrodotoxin in the dose range of 4 to 15 μg/kg. The mean doses which blocked the reticular inhibition of the monosynaptic potential and of the polysynaptic ones were $9{\cdot}0 \pm 1{\cdot}8$ μg/kg and $9{\cdot}0 \pm 1{\cdot}5$ μg/kg respectively. At just below the dose which blocked the reticular inhibition completely, clonic movements of the cats' forelimbs or an increase in rigidity in the shoulders and the forelimbs were frequently observed.

After the reticular inhibition was abolished, reticular stimulation with a higher intensity augmented both reflex potentials slightly as shown in Figure 4. This reticular facilitatory effect was abolished by additional small doses of tetrodotoxin. These results correspond very well with the effects of this drug on intact mice which were reported by Frank & Pinsky (1966) who observed no signs of excitement but convulsions just before the animals died. These results also support the concept that the anaesthetic depression of increase in sodium conductance in the

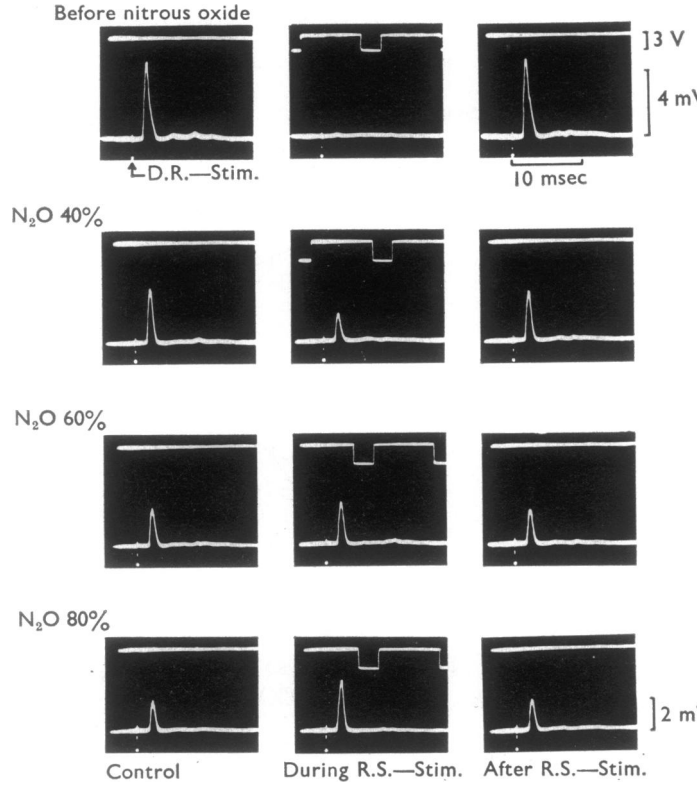

FIG. 2. The effect of nitrous oxide (N_2O) on the reflex potentials before (at the left), during (at the centre) and after reticular stimulation (at the right). The upper line in each recording indicates the reticular stimulation and the lower line contour illustrates the segmental reflex potentials. Dorsal root stimulation (D.R. Stim.) produced a mono- and a few polysynaptic potentials. In this case 60% nitrous oxide completely blocked the reticular inhibition of the reflex potentials and then reticular stimulation with a higher intensity augmented the monosynaptic potential greatly and the polysynaptic ones slightly.

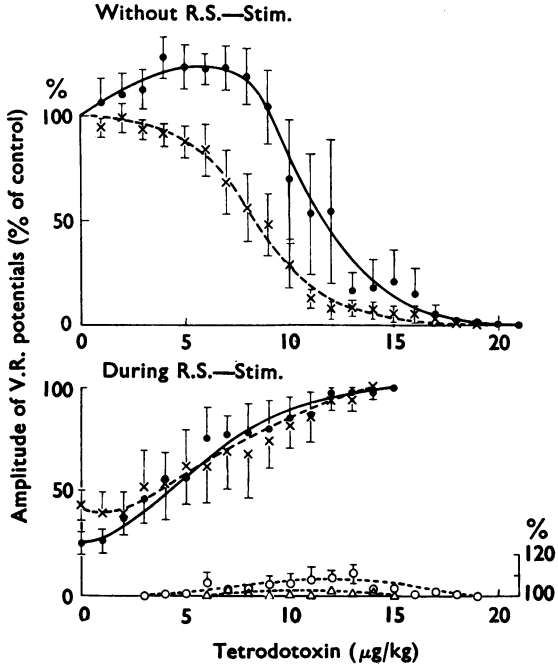

FIG. 3. The effect of tetrodotoxin on the segmental reflex potentials is shown in the upper diagram and that on the reticular inhibition and facilitation is illustrated in the lower diagram as in Figure 1. Each point indicates the mean of 5 observations. Further explanation in the text.

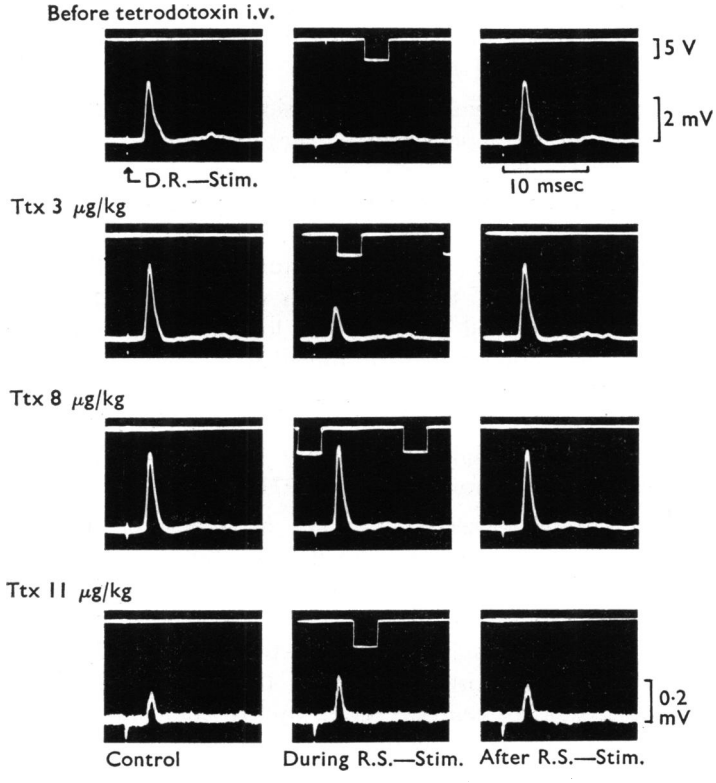

FIG. 4. The effect of tetrodotoxin (Ttx) is shown as in Figure 2. In this case 8 μg/kg of tetrodotoxin abolished the reticular inhibition of both reflex potentials. After abolition of inhibition, reticular stimulation with a higher intensity augmented the reflex potentials although the control reflex potentials were progressively depressed by further doses. Note that the amplification of recording in the lowest rows was 10 times bigger than in the others.

rising phase of the action potentials in certain nerves of the central nervous system may be the most important change at the cellular level in the basic mechanism of the general anaesthesia (Frank, 1968; Frank & Pinsky, 1966; Frank & Sanders, 1963; Thesleff, 1956).

Discussion

The effects of nitrous oxide on the segmental reflex potentials observed in the present investigation as well as the effects of pentobarbital or paraldehyde reported previously (Frank & Ohta, 1971) confirm the observation by DeJong and his co-workers (1968) that the polysynaptic potentials were more resistant to depression by some anaesthetics than were the monosynaptic ones. This shows that the sensitivity of some neural networks to depression by anaesthetics does not depend on the number of synaptic connexions in series in their pathways. On the other hand, the present results obtained with nitrous oxide confirm the hypothesis that the preferential block of the reticulospinal inhibitory pathway may be an important neural mechanism of the excitement stage of general anaesthesia (Frank & Ohta, 1971).

The effect of tetrodotoxin on the central nervous system was first described by Horsburgh, Taturn & Hall (1940). They suggested that the convulsions they observed just before complete paralysis of the limbs were possibly produced by central actions of the drug above the midbrain because anaesthesia or decerebration abolished the convulsions and midthoracic spinal transection eliminated convulsive movements in the hind legs.

Kuriaki *et al.* (1956) investigated the depression of various reflexes by tetrodotoxin and observed that the monosynaptic reflex was more resistant to the toxin than the multisynaptic reflex. In contrast, Koizumi, Lavine & Brooks (1967) reported depression of both monosynaptic and polysynaptic discharges with no difference in susceptibility to tetrodotoxin. However, their investigation was made mainly with anaesthetized cats. The present study confirmed the earlier observations by Kuriaki *et al.* (1956). Furthermore, it was found that the monosynaptic reflex potential was augmented by relatively low doses of tetrodotoxin (up to 9 μg/kg) as previously found with tribromethanol, chloralose or procaine by Frank & Ohta (1971) and with lidocaine by DeJong, Robles & Corbin (1969). This effect is thought to be due to the depression of various inhibitory pathways including the reticulospinal inhibitory pathway (Shimamura, Yamauchi & Aoki, 1968; DeJong *et al.*, 1969; Frank & Ohta, 1971).

Frank & Pinsky (1966) observed no signs of direct central nervous system stimulation by tetrodotoxin and suggested the possibility that suppression of increase in potassium conductance by anaesthetics might be involved in the production of Stage II (the excitement stage) of general anaesthesia. This suggestion cannot be supported by the results obtained in the present study. Thus when the respiration is artificially supported permitting the application of high doses of tetrodotoxin, definite signs of central nervous system stimulation are observed and in our tests of reticular inhibition tetrodotoxin produced effects much like all the other anaesthetics tested.

Hafemann, Costin & Tarby (1969) recently observed seizure-like electrical activity in the lateral geniculate body and the dorsal hippocampus during the recovery from depression by tetrodotoxin. They suggested disrupture of the

TABLE 1. *Effective doses of anaesthetic agents*

			Depression of the reflex potentials (mg/kg)		Blockade of the reticular inhibition (mg/kg)		Abolition of the reticular facilitation
			50%	100%	50%	100%	(mg/kg)
1.	Paraldehyde	Monosynaptic	204±51	440±62	222±15	300±29	392±50
		Poly-	306±33	400±38	206±30	260±41	340±36
2.	Tetrodotoxin	Monosynaptic	13·1±2·0*	19·2±1·5*	5·7±1·2*	9·0±1·8*	17·2±1·5*
		Poly-	8·5±0·8*	13·8±1·7*	7·4±1·4*	9·0±1·5*	12·8±1·8*
3.	Tribromethanol	Monosynaptic	90±19	246±20	35·6±4·0	73·3±3·9	179±16
		Poly-	73±13	204±12	23·9±4·2	58·3±5·0	100±11
4.	Pentobarbital	Monosynaptic	3·8±0·5	45·0±8·0	7·1±1·3	13·6±1·8	33·1±7·6
		Poly-	21·1±7·7	53·6±8·8	3·4±0·5	7·1±1·4	30·0±4·8
5.	Chloralose	Monosynaptic	153±23	204±28	38±7·0	58±10	160±22
		Poly-	103±20	190±28	38±8·0	70±11	148±22
6.	Nitrous oxide	Monosynaptic	57·6±8·6%†	>85%†	51·6±2·4%†	62·0±3·4%†	>85%†
		Poly-	76·2±4·8%†	>85%†	43·0±5·9%†	64·0±4·6%†	>85%†
7.	Procaine	Monosynaptic	108±40	190±58	4·8±1·3	8·5±1·5	161±50
		Poly-	27·4±7·8	144±23	18·8±3·7	52·5±5·5	94±12

* $\times 10^{-3}$. † Concentration % in gas mixture with O_2. The mean effective doses and their standard errors of anaesthetic agents and tetrodotoxin were measured with 5 observations for every drug other than pentobarbital. The mean doses of pentobarbital were calculated from 7 observations except the mean dose abolishing the reticular facilitation which was calculated from 3 observations, because the phenomenon was recognized only in the later experiments. The smaller values indicate a greater effectiveness in depressing the pathways. Note that the values with tetrodotoxin and nitrous oxide are different in scale from the others.

stable steady state activity of a complex structure as the cause of this activity. However, the present results would suggest that the seizure activity is probably produced by depression of inhibitory networks in these structures. Also, similar excessive electrical activities in the pyramidal and/or extrapyramidal systems may be involved in the convulsant movements produced by tetrodotoxin.

The hypothesis was proposed by Matsumoto & Yamamoto (1954) and later confirmed by Furukawa, Sasaoka & Hosoya (1959) that rapidly conducting fibres were more susceptible to tetrodotoxin block than were slowly conducting nerve fibres in toad and frog. This mode of action of tetrodotoxin is different from that of anaesthetic agents which block the smaller nerve fibres more easily (Randt, Collins, Davis & Dillon, 1958; Randt & Collins, 1959) and which therefore, may block the terminal arborizations of nerve fibres especially (Løyning, Oshima & Yokoto, 1964). Nevertheless, the overall effect of tetrodotoxin on the reticulospinal inhibitory pathway appears to be the same as for other anaesthetic agents and this would indicate that the safety factor of transmission in this inhibitory pathway is lower than that in the related excitatory pathway (Furukawa *et al.*, 1959).

To permit a comparison, certain of the results obtained in the present and in the previous study (Frank & Ohta, 1971) are presented in Table 1. Of particular interest is a comparison for the different drugs of the relative doses required to abolish reticular inhibition and reticular facilitation. The smallest ratios (facilitation block: inhibition block) were found for paraldehyde and tetrodotoxin, both noted for their low propensity for producing central nervous system stimulation. And the highest ratio was observed for procaine which readily produces central nervous system stimulation and convulsions.

In Table 1 the drugs have been listed from 1 to 7 in the order of increasing size of this ratio for the monosynaptic potentials (the position of nitrous oxide was estimated from the shape of the facilitation curve (Fig. 1) but of course without further study its position remains pure conjecture). The order of the drugs in this table is in general agreement with an increasing propensity for these drugs to produce central nervous system stimulation and we would suggest that the ability for a general depressant drug to produce central nervous system stimulation is dependent upon its selectivity in depressing inhibitory pathways at lower doses than facilitatory pathways.

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