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Effect of naloxone on rat brain hypoxia

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Opiate antagonists have been indicated in haemorrhagic shock (Faden & Holaday 1979), mainly because of their cardiovascular properties (Feuerstein et al 1981). Naloxone stimulates respiratory activity by enhancing bulbar sensitivity to carbon dioxide possibly through an enkephalinergic mechanism (Denavit-Saubié et al 1978, Beubler 1980; Isom & Elshowihy 1982). Shock and severe hypotension often result in ischaemic brain hypoxia (Kovach & Sandor 1976). Our main objective has been to find out if this protective effect of naloxone could be attributed to an antihypoxic mechanism. For this purpose we chose a method that to a large extent reduced cardiovascular influence (Rossignol et al 1982) and suppressed respiratory interference.

Method

The rats were either artificially ventilated (0·01 ml g^{-1} s^{-1}) before pneumothorax or allowed to breathe spontaneously as indicated in Table 1. The number of ischaemic episodes tolerated before death by cardiac arrest directly indicated the overall antihypoxic protective effect. On the other hand, the number of ischaemic episodes before completion of electrical cerebral silence allowed evaluation of functional protection.

Results

Table 1 shows that there was a significant increase in the number of ischaemic episodes tolerated before death, by artificially ventilated controls compared with the spontaneously breathing control rats.

Naloxone (20 μg i.c.v. per animal) showed no favourable effects, either in the spontaneously breathing or the artificially ventilated group in whom it appeared deleterious.

Table 1. Number of ischaemic episodes tolerated (m ± s.e.m. n=6).

	Before e.e.g. silence		Before death	
	S.B.	A.V.	S.B.	A.V.
Control	5·33 ±0·55	5·83 ±0·47	7·00 ±0·73	10·80 ±0·61
Naloxone 20 μg i.c.v.	4·66 ±0·76	5·00 ±0·44	6·50 ±0·85	8·00** ±0·57
Naloxone 70 μg i.c.v.	4·83 ±0.40	3·66** ±0·33	7·00 ±0·25	14·66** ±1·11

S.B. = spontaneous breathing, A.V. = artificial ventilation, * = P <0·02, **= <0·01 versus control.

Conversely, given at 70 μg i.c.v. per animal, with artificial ventilation, naloxone increased the number of ischaemic episodes before death to 14·66 ± 1·11, while in the same conditions, the number of episodes tolerated before electrical silence was only 3·66 ± 0·33, that is, even fewer than the number after the small dose of naloxone. In the spontaneously breathing group, naloxone at 70 μg i.c.v. per animal gave results that were not statistically different from those after 20 μg.

Discussion

The simplest explanation seems to be that naloxone is able to induce favourable effects only in pathological states where there is an increased release of endogenous opiates as in haemorrhagic shock. However, the high dose of 70 μg effectively protects against cardiovascular death but has no protective effect against the disappearance of e.e.g.; it also significantly shortens the delay before onset of silence, at least under artificial ventilation.

As in these circumstances, the time of death is shortened by cardiac arrest, the effect is largely one of cardiovascular protection (Feuerstein et al 1981). Following such an assumption, the reality of some haemorrhagic shock components being present after the ischaemic episodes must be recognized.

Another explanation could take into consideration the non-enkephalinergic effects of large doses of naloxone. Indeed, Feldberg et al (1982) reported strong hyperglycaemia in the cat, especially after i.c.v. administration of doses of naloxone of a similar size and, it has long been known that hyperglycaemia can protect against hypoxic effects (Hershgold & Riley 1959; Henry et al 1974).

However, enkephalinergic transmission does not intervene in the genesis of central silence, at least in our experimental conditions.

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A possible site of action of nicotine in the bronchial smooth muscle preparation of guinea-pig

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The bronchioles are innervated by excitatory cholinergic parasympathetic and inhibitory adrenergic sympathetic nerve fibres. Ganglion cells exist in the lung distributed along the branches of the bronchial tree (Macklin 1929). Actions of nicotine on the guinea-pig isolated trachea are well known (Hawkins & Paton 1958; Chiou & Long 1969; Jones et al 1980) but only Hawkins & Paton (1958) have reported on the action of nicotine on the isolated bronchial muscle of guinea-pig.

Male guinea-pigs, 350 to 400 g, were killed by a blow on the head and main bronchi isolated and cut helically. The preparations (2 × 30 mm) were suspended in a 20 ml organ bath filled with a physiological solution (NaCl 118, KCl 4·72, $CaCl_2$ 2·56, $MgSO_4.7H_2O$ 0·16, KH_2PO_4 1·20, $NaHCO_3$ 25·0 and dextrose 10·0 mM) gassed with carbogen and kept at 32 °C. Responses to drugs were recorded isometrically under a tension of 0·5 g. In some experiments, two platinum electrodes (2 mm × 35 mm) were placed 5 mm apart and field stimulation of the bronchial preparations was carried out by passing a rectangular pulse of 0·5 ms duration, supramaximal voltage and a frequency of 10 Hz between the two electrodes for 10 s. The experiments were started after the preparations had developed their spontaneous tone for 60 min. All agonists were applied to the preparation at intervals of 60 min. The concentration of cyclic (c) GMP was measured by the methods of Steiner et al (1972) to estimate the effects of nicotine and acetylcholine on a tissue concentration of cGMP. Two pieces of bronchus were prepared. One was used for measuring the control concentration of cGMP and the other for any change after exposure to a drug. Protein concentration was measured by the method of Lowry et al (1951), with bovine serum albumin as the standard.

Contractile response to nicotine was reproducible under the conditions used. Nicotine (10^{-6}–10^{-3} M) contracted the bronchial smooth muscle concentration-dependently (Fig. 1). The maximum response to nicotine was 38·5 ± 4·0% (mean ± s.e.m. of 6 experiments) of that to acetylcholine. No inhibitory response to nicotine (10^{-6}–10^{-3} M) was observed in any preparation used; all were greatly relaxed by 10^{-4} M papaverine (Fig. 1). In the following experiments, nicotine 10^{-4} M and the equieffective acetylcholine 10^{-4} M were used as agonists. Ganglion blockers, hexamethonium (10^{-5} M) and pentolinium (10^{-6} M) at concentrations that were enough to inhibit the concentration action curve of nicotine in the guinea-pig ileum (Van Rossum 1962), considerably reduced the contractile response to nicotine. But this was not influenced by 5 min treatment with atropine (10^{-6} M) which almost inhibited the responses to acetylcholine 10^{-4} M (Table 1). Fifteen min treatment of the bronchus with tetrodotoxin (3 × 10^{-6} M) also was without any effect on the response to nicotine. Furthermore, SX-284 (2-(1,2-benzisoxazol-3-yl)-3-[2-(2-piperdinoethoxy)phenyl]acrylonitrile) (3 × 10^{-7} M), which inhibits acetylcholine release from the parasympathetic nerve specifically (Takayanagi et al 1982), did not influence the nicotine-induced contraction. Field stimulation induced a contractile response

Table 1. Effects of some drugs on the contractile responses to nicotine, acetylcholine and field stimulation. Each value is presented as a mean ± s.e.m. of 6 experiments. ( ): incubation time. SX-284 is an inhibitor of acetylcholine release from parasympathetic nerves (Takayanagi et al 1982).

Treatment	% of contraction
Nicotine, 10^{-4} M	100·0
+ hexamethonium, 10^{-5} M (5 min)	32·7 ± 2·8*
+ pentolinium, 10^{-6} M (5 min)	17·0 ± 3·5*
+ atropine, 10^{-6} M (5 min)	95·0 ± 8·1
+ tetrodotoxin, 3 × 10^{-6} M (15 min)	99·7 ± 11·4
+ diphenhydramine, 10^{-6} M (5 min)	94·8 ± 7·6
+ indomethacin, 10^{-6} M (30 min)	112·3 ± 15·6
+ SX-284, 3 × 10^{-7} M (15 min)	93·6 ± 8·3
+ physostigmine, 10^{-6} M (30 min)	103·6 ± 7·6
Acetylcholine, 10^{-4} M	100·0
+ atropine, 10^{-6} M (5 min)	12·4 ± 4·9*
+ physostigmine, 10^{-6} M (30 min)	229·3 ± 19·3*
Field stimulation	100·0
+ tetrodotoxin, 3 × 10^{-6} M (15 min)	15·7 ± 4·8*
+ physostigmine, 10^{-6} M (30 min)	147·7 ± 5·9*

* Significant difference from 100% at $P < 0.05$.

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