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EFFECTS OF KETAMINE ON THE PERIPHERAL AUTONOMIC NERVOUS SYSTEM OF THE RAT

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1 The effects of ketamine (2-(*o*-chlorophenyl) 2-methylaminocyclohexanone) (2–50 mg/kg) on the responses of the pithed rat arterial pressure, anococcygeus muscle and colon to selective stimulation of the spinal autonomic outflows were examined.

2 Ketamine depressed the vasopressor response produced by stimulation of the lumbar sympathetic outflow in a dose-dependent manner but did not significantly affect the pressor response to intravenous noradrenaline (NA) administration.

3 Ketamine depressed the motor responses of the anococcygeus to stimulation of the pre-ganglionic lumbar sympathetic outflow or to stimulation of post-ganglionic fibres in the sacral region in a dose-dependent manner, the response to preganglionic stimulation being relatively more sensitive to such depression. The anococcygeus response to NA was significantly potentiated with doses of ketamine of 20 mg/kg and 50 mg/kg.

4 Ketamine depressed the motor response of the smooth muscle of the colon to stimulation of the sacral parasympathetic outflow in a dose-dependent manner and at lower doses than were required to produce an equivalent depression of the sympathetic responses in the other tissues.

5 A comparison was made of the effects of ketamine and cocaine on the motor responses of the anococcygeus muscle *in vitro* to NA, carbachol and field stimulation. Both ketamine and cocaine produced a non-specific depression of all responses at high doses whereas cocaine but not ketamine produced a large potentiation of NA and motor nerve responses at lower doses.

6 The results are discussed in relation to the hypothesis that ketamine might elevate blood pressure in conscious animals and man by potentiating vascular adrenergic responses.

Introduction

Ketamine is unique among intravenous anaesthetic agents in producing an increase in blood pressure and heart rate in man and in several species of animals (Domino, Chodoff & Corssen, 1965; McCarthy, Chen, Kaump & Ensor, 1965). It has been suggested that the pressor action involves the sympathetic nervous system since it can be abolished by pre-treatment with α-adrenoceptor blocking agents (Traber & Wilson, 1969), but it is not known whether the site of action is central or peripheral. Chang, Chan & Ganendran (1969) have suggested that ketamine may release catecholamines into the bloodstream in the pithed rat and that this might be responsible for its pressor action.

Recently Nedergaard (1973), and Montel, Starke, Gorlitz & Schumann (1973) have shown that ketamine can inhibit the neuronal uptake mechanism for noradrenaline (NA) *in vitro*, and can potentiate responses to both NA and sympathetic nerve stimulation both *in vitro* and with prolonged infusion of ketamine *in vivo*. The neuronal uptake process for NA has been shown to be an important factor in the physiological operation of adrenergic nerves and in the pharmacological efficacy of many drugs *in vitro* or in the central nervous system *in vivo* (Iversen, 1973) but has not until now been implicated in the peripheral actions of drugs *in vivo*. However, Muscholl (1961) has demonstrated that cocaine blocks neuronal NA uptake and potentiates pressor responses to NA in the pithed rat.

In this study, the effects of ketamine were examined on the responses of the arterial blood pressure and the anococcygeus muscle to sympathetic nerve stimulation or to intravenous NA and on the

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responses of the colon to parasympathetic nerve stimulation, in the pithed rat (Gillespie, MacLaren & Pollock, 1970).

In order to compare the doses used with those used previously to block neuronal NA uptake, the effect of ketamine was also examined on the responses of the anococcygeus muscle *in vitro* to agonist drugs and to field stimulation. A preliminary account of these results has been published (Clanachan & McGrath, 1975).

Methods

Sprague-Dawley rats (250–300 g) were used.

Pithed rat

The rats were pithed under halothane by the method of Gillespie *et al.* (1970). The trachea was intubated and ventilation with 100% O_2 at 60 min^{-1} (Harvard Instruments Rodent Ventilator) was adjusted to give an end tidal CO_2 of 4% (Beckman LB2 Medical Gas Analyser). Drugs were administered via a polythene cannula in the right jugular vein and arterial pressure monitored from a polythene cannula in the left carotid artery (Bell and Howell type 4-327-L221 transducer).

The tension in the anococcygeus muscle was monitored isometrically (Devices type 4151 transducer), by tying a thread around the ventral bar of the tissue via an incision in the scrotum. The tissues were kept moist by a pool of liquid paraffin (Gillespie & McGrath, 1973). Activity of the circular muscle of the colon was monitored by placing a small balloon inside and measuring pressure changes with a Bell and Howell (type 4-327-L221) transducer (Gillespie *et al.*, 1970). Rectal temperature was maintained at 37°C by warming the animal with a tungsten lamp. Anococcygeus tension, colon pressure, heart rate and arterial pressure were displayed on a Devices MX4 recorder.

Stimulation of the sympathetic outflow was by means of the unshielded 10 mm long stainless steel tip of the pithing rod and a silver indifferent electrode inserted under the skin (Devices isolated stimulator, supramaximal voltage, 1 ms pulses, 10 Hz, 10 s periods every 5 minutes). Stimulation at L1-2 produced pressor responses due to stimulation of the preganglionic sympathetic outflow to the vascular bed (Gillespie *et al.*, 1970), and contraction of anococcygeus muscles due to stimulation of the preganglionic sympathetic outflow to this latter tissue (Gillespie & McGrath, 1973). Stimulation at S1-2 produced no pressor response but contracted the anococcygeus muscles due to stimulation of postganglionic fibres to this tissue (Gillespie & McGrath, 1973), and contracted the circular muscle of the colon due to simultaneous stimulation of the preganglionic sacral parasympathetic outflow (Gillespie *et al.*, 1970). Contraction of skeletal muscle was prevented by administration of pancuronium (1 mg/kg).

Standard, reproducible, submaximal responses were obtained by stimulation of the appropriate region of the spinal canal and by the administration of NA (i.v.) at 10 min intervals. Doses of ketamine were then administered intravenously 2 min before a stimulation period or NA dose was due. At least 30 min was allowed between doses of ketamine for return of responses to control levels. Each response following ketamine was expressed as a percentage of the control response preceding ketamine.

Anococcygeus in vitro

Rats were killed by a blow on the head, and exsanguination. Isolated anococcygeus muscles were studied *in vitro* by the method of Gillespie (1972); the muscles were placed in 10 ml baths containing Krebs solution at 37°C and gassed with 95% O_2 and 5% CO_2. The muscles were threaded through platinum field stimulation electrodes and tension recorded isometrically with Grass FTO3 transducers. Tension was displayed on a Grass Model 7 polygraph. Field stimulation was applied with a Tektronix stimulator (supramaximal voltage, 1 ms pulses) and drugs added to the bath in volumes of 0.2 ml or less. Reproducible submaximal responses were obtained to field stimulation at 10 Hz for 10 s periods or to addition to the bath of NA 3 μM or carbachol 1 μM. Sufficient contact time was allowed with the agonist drugs to reach a plateau response. These parameters were chosen since they produce responses of similar magnitude; approximately 50% of the maximum response of the tissue. When each concentration of ketamine and cocaine was added to the bath, at least 5 min was allowed for equilibration before responses to NA, carbachol or field stimulation were tested. Following each dose, the tissue was washed until reproducible responses similar to the initial controls were obtained before testing the next highest dose of ketamine or cocaine. Responses in the presence of each dose of ketamine or cocaine were expressed as a percentage of the control preceding administration at that dose.

Comparison of responses before and after each dose of ketamine or cocaine *in vitro* or in the pithed rat was made using a paired *t*-test.

The following drugs were used: carbachol (Burroughs Wellcome); cocaine hydrochloride (Macarthys); ketamine hydrochloride (Ketalar, Parke Davis); noradrenaline bitartrate (Sigma) and pancuronium bromide (Pavulon, Organon).

Results

Pithed rat arterial pressure responses

Ketamine 2–50 mg/kg had a biphasic effect on the resting arterial pressure of the pithed rat, producing an

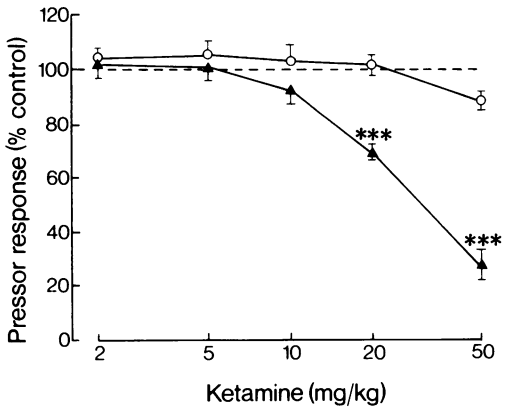

Figure 1 Effect of ketamine on the arterial pressure response in the pithed rat to intravenous noradrenaline (O) or stimulation of the preganglionic sympathetic outflow at L1-2 (▲). NA dose 400 ng/kg; sympathetic stimulation, 10 mm electrode, supramaximal voltage, 1 ms pulses, 10 Hz for 10 s period. Responses 2 min after ketamine are expressed as a percentage of the control response preceding ketamine. Vertical lines show s.e. mean ($n=6$).

Statistical comparison using paired t test of post-ketamine responses compared with controls: *** $P<0.001$.

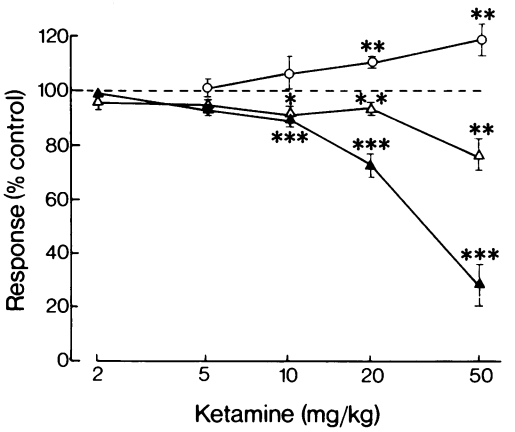

Figure 2 Effect of ketamine on the anococcygeus muscle response in the pithed rat produced by intravenous noradrenaline (O), stimulation of the preganglionic sympathetic outflow at L1-2 (▲) or stimulation of postganglionic sympathetic fibres at S1-2 (△). NA dose 4 μg/kg; sympathetic stimulation, 10 mm electrode, supramaximal voltage, 1 ms pulses, 10 Hz for 10 s period. Responses 2 min after ketamine are expressed as a percentage of the control response preceding ketamine. Vertical lines show s.e. mean ($n=6$).

Statistical comparison using paired t test of post-ketamine responses compared with controls: ** $0.01>P>0.001$; *** $P<0.001$.

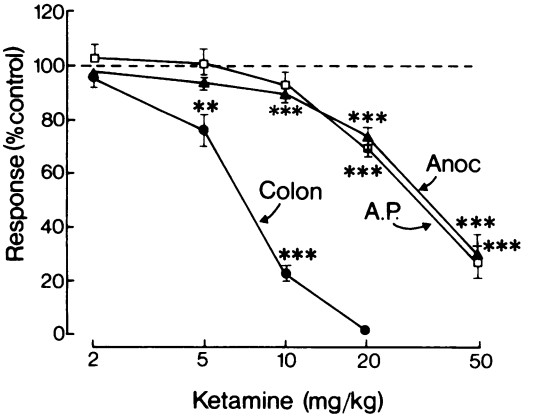

Figure 3 Effect of ketamine on the responses in the pithed rat to stimulation of (1) the sympathetic outflow at L1–2; arterial pressure (A.P., □) and anococcygeus (Anoc, ▲); (2) the parasympathetic outflow at S1–2; colon (Colon, ●). Stimulation parameters: 10 mm electrodes, supramaximal voltage 1 ms pulses; in (1) 10 Hz, 10 s period, in (2) 10 Hz, 30 s period. Responses 2 min after ketamine are expressed as a percentage of the control response preceding ketamine. Vertical lines show s.e. mean ($n=6$).

Statistical comparison using a paired t test of post-ketamine responses cf. controls: ** $0.01>P>0.001$; *** $P<0.001$.

initial transient fall in pressure followed by a short-lived rise. The initial fall was accompanied in every case by a fall in heart rate.

NA (400 ng/kg; i.v.) produced reproducible submaximal rises in arterial pressure of 65 ± 3 mmHg, $n=20$. Ketamine 2–50 mg/kg had no significant effect on these responses (Figure 1).

Stimulation of the preganglionic sympathetic outflow (Ll-2) at 10 Hz for 10 s produced reproducible, submaximal rises in arterial pressure of 37 ± 3 mmHg, $n=20$. Ketamine depressed these responses in a dose-dependent fashion which was statistically significant at 20 and 50 mg/kg (Figure 1).

Anococcygeus responses

Ketamine 2–50 mg/kg had no effect on the resting tension of the anococcygeus muscle.

NA (4 μg/kg; i.v.) caused reproducible submaximal increases in anococcygeus tension of 2.9 ± 0.2 g, $n=20$. Ketamine produced a dose-dependent potentiation of these responses which was statistically significant at 20 and 50 mg/kg (Figure 2).

Stimulation of the preganglionic sympathetic outflow (L1-2) at 10 Hz for 10 s produced reproducible, submaximal increases in anococcygeus tension of 6.6 ± 0.2 g, $n=20$. Ketamine produced a

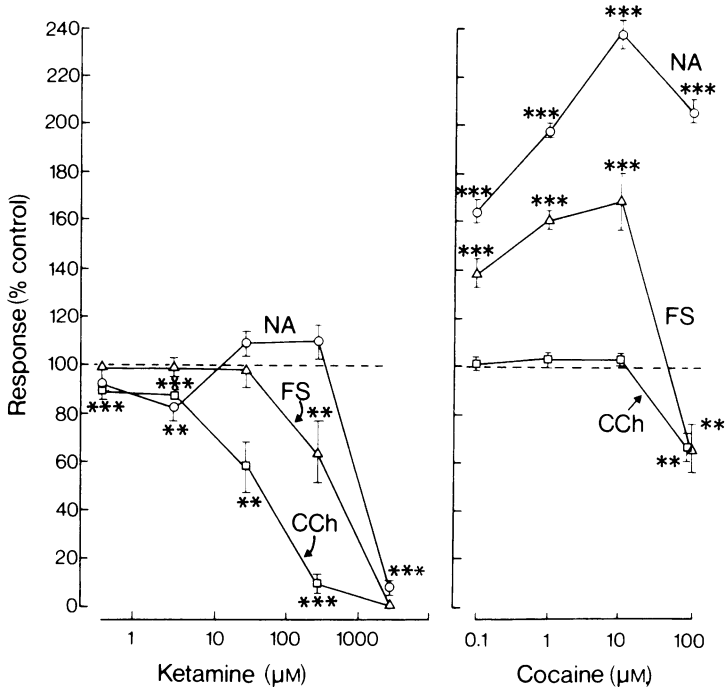

Figure 4 Effects of ketamine and cocaine on the motor responses of the *in vitro* anococcygeus muscle, to noradrenaline (NA) 3 μM (○), carbachol (CCh) 1 μM (□) and field stimulation (FS, △). Responses in the presence of ketamine or cocaine are expressed as a percentage of control responses. Vertical lines show s.e. mean ($n=6$).

Statistical comparison using a paired t test of responses in the presence of ketamine cf. controls: ** $0.01 > P > 0.001$; *** $P < 0.001$.

dose-dependent inhibition of these responses which was statistically significant at 10 mg/kg and above (Figure 2). Stimulation with the pithing rod electrode at Sl-2, (10 Hz, 10 s) which excites postganglionic sympathetic fibres to the anococcygeus (Gillespie & McGrath, 1973), produced reproducible submaximal responses in the anococcygeus of 6.4 ± 0.3 g, $n=20$. Ketamine inhibited these responses in a dose-dependent manner which was statistically significant at 10 mg/kg and above. The extent of this inhibition was significantly less than in the case of preganglionic stimulation (Figure 2).

Colon responses

Stimulation of the parasympathetic outflow at L5-6 produced reproducible increases in pressure in a balloon inside the colon. Ketamine inhibited these responses in a dose-dependent manner which was statistically significant at doses of 5 mg/kg and above. With a dose of 20 mg/kg of ketamine the response was completely extinguished in every experiment (Figure 3).

Anococcygeus in vitro

Effects of ketamine. The dose-response curve in Figure 4 illustrates that ketamine (0.3 μM to 3 mM) inhibited responses to all three stimuli (NA, carbachol and field stimulation) in a dose-related manner but the magnitude of this inhibition was carbachol > field stimulation > NA. In addition there was a relative potentiation of the responses to NA in the range of concentration of ketamine 30 μM to 300 μM compared with the inhibition produced at lower or higher doses although this did not reach statistical significance compared with the pre-ketamine controls. In 7 out of 8 tissues tested, however, the response to NA was potentiated by either 30 μM or 300 μM ketamine.

Effects of cocaine. Cocaine significantly potentiated responses to NA and field stimulation with a maximum at 10 μM, whereas responses to carbachol were not significantly increased at any dose tested (Figure 4). The potentiation of NA responses increased to a maximum at cocaine 10 μM and declined with a further increase in cocaine concen-

tration to 100 μM. The responses to carbachol were unaffected by cocaine at doses up to 10 μM, whereas they were depressed at 100 μM. Responses to field stimulation were potentiated by cocaine in a dose-related manner until the cocaine concentration reached 10 μM. At cocaine 100 μM, responses to field stimulation were significantly inhibited (Figure 4).

Discussion

These results lend little support to the hypothesis that ketamine might raise blood pressure by peripheral potentiation of sympathetic nerve responses. The only evidence found in this study for a potentiation of adrenergic responses *in vivo* by ketamine was a small increase in the response of the anococcygeus muscle to NA with the highest doses of ketamine of 20 or 50 mg/kg (i.v.). However, the response of the vasculature to NA showed no potentiation at any dose and the responses of both the vasculature and the anococcygeus muscle to stimulation of the pre-ganglionic sympathetic outflow were depressed by ketamine in a dose-dependent manner. *In vitro,* the response to NA of the anococcygeus muscle was depressed in a dose-dependent manner although in the range of ketamine concentration 30 μM to 300 μM, the responses of some preparations were increased, perhaps indicating a small potentiating effect of ketamine in this range. The response to field stimulation of the adrenergic motor nerves in the anococcygeus was, however, also depressed by ketamine in a dose-dependent manner.

This does not conflict with the evidence that ketamine can block neuronal NA uptake *in vitro* (Nedergaard, 1973; Montel *et al.,* 1973). However, at the high doses of ketamine required to produce potentiation of NA responses, widespread depressant effects contribute to a net effect in the whole animal which would act against any pressor effect. Thus the responses to stimulation of post-ganglionic sympathetic nerves to the anococcygeus are depressed *in vivo* or *in vitro* and responses to preganglionic sympathetic nerve stimulation to the anococcygeus or the vascular system are depressed to an even greater degree. This suggests depressant effects at two sites at least in the sympathetic pathway *viz.* the ganglion synapse and the postganglionic terminal synapse. When the parasympathetic pathway to the colon is considered, the degree of depression by ketamine was greater still and occurred at doses of ketamine as low as 5 mg/kg (i.v.) (Figure 3).

The effects of cocaine on the *in vitro* anococcygeus illustrate that with the protocol employed it is possible to detect potentiation of adrenergic responses due to blockade of neuronal uptake of NA. It has previously been demonstrated that cocaine 1 μM potentiates NA responses in this tissue (Gibson & Pollock, 1973). The present study confirms this and illustrates that this effect increases with increasing doses of cocaine up to 10 μM as also does the potentiation of the effects of adrenergic nerve stimulation. At 100 μM cocaine, however, the local anaesthetic action predominates and both nerve-mediated and post-synaptic responses are depressed. *In vivo,* Muscholl (1961) has shown that cocaine (10–20 mg/kg) can potentiate the pressor response to NA in the pithed rat and McGrath (1973) has shown that cocaine (1 mg/kg) can potentiate the vasopressor and anococcygeus muscle responses to stimulation of the lumbar sympathetic outflow of the pithed rat. Simpson (1975) has shown also that the ID_{50} for blockade of neuronal NA uptake into the heart of the pithed rat is 3 mg/kg cocaine.

The relative potency of ketamine and cocaine at blocking neuronal NA uptake is relevant to any possible mechanism contributing to a pressor response in the whole animal. From the present study and from the earlier work on blockade of neuronal NA uptake (Nedergaard, 1973; Montel *et al.,* 1973) ketamine appears to be 30 to 100 times less potent than cocaine on a molar basis at potentiating NA responses. If, therefore, 1–20 mg/kg (3.3–66 μmol/kg) cocaine is required to produce potentiation of responses *in vivo,* it is likely that the dose of ketamine to produce the same effect would be at least in the order of 20–100 mg/kg (89–440 μmol/kg). This is confirmed by the results for the anococcygeus in the pithed rat where only doses of ketamine of 20 and 50 mg/kg (i.v.) produced potentiation of NA responses and any higher doses stopped the heart by a direct depression of the myocardium (Dowdy & Kaya, 1968). In contrast, the anaesthetic doses of ketamine which raise arterial pressure in man and several animal species are of the order of 2–5 mg/kg (i.v.) (McCarthy *et al.,* 1965; Domino *et al.,* 1965; Traber & Wilson, 1969; McGrath, Mackenzie & Millar, 1975a). In addition, since ketamine does not block the baroreceptor reflex controlling sympathetic tone (McGrath, Mackenzie & Millar, 1975b), any small effect due to a peripheral action would not be maintained due to the 'buffering' effect of this reflex.

We suggest that ketamine, like cocaine, possesses the dual properties of neuronal NA uptake blockade and local anaesthetic-type depression of synaptic transmission, but that whereas cocaine possesses the former property at low doses and the latter at high doses, with ketamine the optimal doses for each property are close together and, therefore, the net effect will depend on the system under investigation.

In conclusion, the results suggest that ketamine does not produce pressor effects in anaesthetic doses by a potentiation of adrenergic responses. Doses which do produce some potentiation of peripheral adrenergic responses are higher than anaesthetic doses and produce predominantly depressant effects on the peripheral autonomic nervous system.

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