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INHIBITION OF REFLEX VAGAL BRADYCARDIA BY A CENTRAL ACTION OF 5-HYDROXYTRYPTOPHAN

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1 Vagally mediated reflex bradycardia was elicited in spinal cats with intravenous pressor doses of noradrenaline. Administration of 5-hydroxytryptophan (1.5 and 3 mg total dose) into the fourth cerebral ventricle reduced the reflex bracycardia.

2 Inhibition of central amino acid decarboxylase with R044602 prevented the effects of 5-hydroxytryptophan. After intravenous administration of 5-hydroxytryptophan, vagal reflex bradycardia was not affected.

3 Results suggest that 5-hydroxytryptophan acts in the CNS to inhibit baroreceptor-mediated vagal reflex bradycardia and that action is mediated via conversion to 5-hydroxytryptamine.

Introduction

Previous studies indicated that the 5-hydroxytryptamine (5-HT) precursor, 5-hydroxytryptophan (5-HTP), acts in the central nervous system to reduce sympathetic outflow (Baum & Shropshire, 1975; Tadepalli, Mills & Schanberg, 1977), arterial pressure, heart rate and decreases carotid occlusion pressor response in cats and dogs (McCubbin, Kaneko & Page, 1960; Florez & Armijo, 1974; Antonaccio & Robson, 1975; Baum & Shropshire, 1975; Tadepalli *et al.*, 1977). It is suggested that the action of 5-HTP is mediated via conversion to 5-HT since inhibition of central L-amino acid decarboxylase prevented the actions of the precursor (Florez & Armijo, 1974; Tadepalli *et al.*, 1977). In agreement with this are the observations that 5-HT, injected into the cerebral ventricles, reduced arterial pressure and carotid occlusion pressor response (Bhargava & Tangri, 1959; McCubbin *et al.*, 1960; Dhawan, Dhawan & Gupta; 1967). On the other hand, studies in rats showed that 5-HT injected into the cerebral ventricles (Lambert, Friedman & Gershon, 1975; Krstic & Djurkovic, 1976; Lambert, Friedman, Buchweitz & Gershon, 1978) or into the anterior hypothalamus (Smits & Struyker-Boudier, 1976) elicited a sympathetic mediated pressor response, accompanied by both tachycardia and bradycardia (Lambert *et al.*, 1978). These studies did not elaborate the effects of central 5-hydroxytryptaminergic systems on the reflex vagal activation elicited by stimulating systemic baroreceptors. In the present study, the effects of intracerebroventricularly administered 5-HTP on vagally mediated cardiac responses were investigated in spinal cats.

Methods

Cats weighing 2.0 to 3.5 kg were anaesthetized with a mixture of α-chloralose (55 mg/kg) and urethane (250 mg/kg) administered intraperitoneally. Arterial pressure was measured from a femoral arterial cannula with a Statham P-23 Ac transducer and recorded on a Grass model 7 polygraph. Mean arterial pressure was calculated by the formula: diastolic pressure + 1/3 pulse pressure. Heart rate was measured with a tachograph preamplifier (7P44B) triggered by the arterial pressure pulse. A femoral vein was cannulated for the administration of drugs. The trachea was cannulated and the animals were artificially ventilated.

Injection into fourth cerebral ventricle (4CV)

The cat's head was fixed in a stereotaxic instrument (David-Kopf) and a cannula was inserted 2 to 4 mm rostral to the obex in the midline so that the tip was just above the floor of the 4CV (Tadepalli *et al.*, 1977). Drugs were injected in a volume of 0.1 to 0.3 ml of Ringer-Locke solution and the cannula was flushed with 0.07 ml of the solvent. Total volume of 0.17 to 0.37 ml was injected over a period of 5 to 8 min. The same volume of Evans blue was injected at the end of the experiment and most of the dye was detected in the 4CV. There was also evidence of colouration in the spinal canal and on cut surface of the spinal cord.

Spinal transection

The spinal cord was exposed at the atlanto-occipital interspace; 0.05 to 0.1 ml of 2% lidocaine was injected

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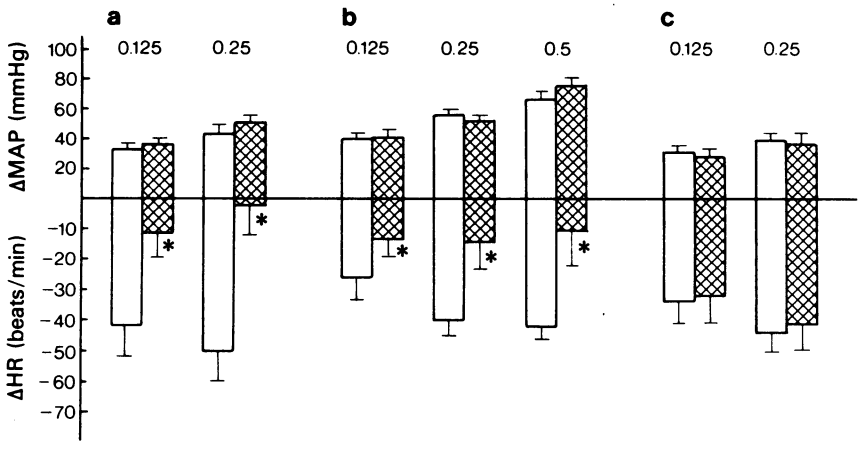

Figure 1 Effect of intracerebroventricular 5-hydroxytryptophan (5-HTP) on intravenous noradrenaline-induced pressor and heart rate responses. Doses of noradrenaline are given at the top of the columns. Open columns: before; hatched columns: after 5-HTP. (a) 5-HTP 1.5 mg ($n = 5$); (b) 5-HTP 3.0 mg ($n = 5$); (c) 5-HTP 3.0 mg after R04-4602 ($n = 5$). After both 1.5 and 3.0 mg doses of 5-HTP, pressor responses to intravenous noradrenaline were not altered while the bradycardia was significantly reduced. R04-4602 pretreatment prevented the effect of 5-HTP.

directly into the spinal cord just below the transection level and the spinal cord was transected at the C1 level.

Drugs

The following drugs were used: α-chloralose (Aldrich Chemical Company, Milwaukee, Wisconsin), ethyl carbamate (urethane, Sigma, St. Louis, Missouri), noradrenaline bitartrate (levophed, Winthrop Laboratories, New York), L-5-hydroxytryptophan (Calbiochem, San Diego, California), DL-serine-2-[(2,3,4-trihydroxyphenyl) methyl] hydrazine hydrochloride (R04-4602 Hoffmann-LaRoche, Nutlay, New Jersey), lidocaine (xylocaine hydrochloride, Astra Pharmaceutical, Worcester, Massachusetts).

Statistical analysis

All data are presented as mean ± s.e. Data were analyzed by a paired t test and Student's t test was used for comparison between groups. All differences were considered significant at $P < 0.05$.

Results

Effects of intracerebroventricular (i.c.v.) 5-hydroxytryptophan on resting arterial pressure and heart rate

There was no appreciable change in resting arterial pressure and heart rate during the first hour after administering 5-HTP but both the variables were decreased during the second hour reaching maximum between 90 and 120 min. The decrease in arterial pressure was not significant (from 66 ± 4 to 57 ± 4 mmHg after 1.5 mg, and from 70 ± 5 to 64 ± 7 mmHg after 3 mg dose of 5-HTP). Heart rate decreased from 129 ± 6 to 101 ± 11 beats/min after 1.5 mg and from 133 ± 10 to 110 ± 11 beats/min after 3 mg dose of 5-HTP ($P < 0.05$). The resting cardiovascular variables remained low for up to 180 to 200 min following both doses of 5-HTP.

Effect of i.c.v. 5-hydroxytryptophan on reflex vagal bradycardia elicited by noradrenaline

In spinal cats with intact vagi, reflex bradycardia was evoked by intravenous pressor doses (0.06 to 0.5 μg/kg) of noradrenaline and were repeated at least once more before administration of 5-HTP into the 4CV. In different groups of cats effects of 1.5 and 3.0 mg total doses of 5-HTP were examined and the results are shown in Figure 1. Following both 1.5 mg (a) and 3.0 mg (b) doses of 5-HTP, reflex bradycardia responses were significantly depressed ($P < 0.05$). The pressor responses to intravenous noradrenaline were not altered by i.c.v. 5-HTP. It should be mentioned that in some experiments there was an increase in the reflex bradycardia responses immediately following 5-HTP, but the effect was transient and depression of the reflex responses occurred subsequently. Maximal depression of the reflex heart rate responses occurred within 90 min following the administration of 5-HTP and there was no significant difference between the two doses. In some experiments intravenous

noradrenaline-induced bradycardia was changed to tachycardia after i.c.v. 5-HTP. Depression of the reflex bradycardia was observed before maximal changes in resting cardiovascular variables occurred.

Effects of R04-4602 on the actions of i.c.v. 5-hydroxytryptophan

In a series of 5 experiments, effects of i.c.v. 5-HTP were examined following administration of a central L-amino acid decarboxylase inhibitor, R04-4602. A total dose of 25 mg of R04-4602 (in 0.1 ml of Ringer-Locke solution) administered into the 4CV did not affect the pressor or reflex bradycardia induced by intravenous noradrenaline. 5-HTP (3 mg, 4CV) administered 60 to 80 min after R04-4602 failed to reduce the reflex bradycardia during a 2 h observation period (Figure 1c). In fact, slight enhancement of the reflex responses were observed in some experiments. 5-HTP did not significantly alter the resting cardiovascular variables in R04-4602 pretreated cats. Thus arterial pressure was changed from 64 ± 1 to 72 ± 5 mmHg and heart rate from 140 ± 13 to 136 ± 13 beats/min after 5-HTP.

Effects of intravenous 5-hydroxytryptophan

In order to test the possibility that the effects of 5-HTP injected into 4CV were not due to a peripheral action of the drug, effects of intravenous 5-HTP (3 mg) were examined in a separate group of 3 cats. Intravenous 5-HTP had no significant effect on pressor or reflex bradycardia induced by intravenous noradrenaline during a 2 h observation period. Thus noradrenaline at doses of 0.125 and 0.25 μg/kg (i.v.) elicited pressor responses of 34 ± 7 and 50 ± 8 mmHg associated with bradycardia of 25 ± 9 and 40 ± 16 beats/min respectively. Following intravenous 5-HTP, the pressor responses to same intravenous doses of noradrenaline were 38 ± 8 and 55 ± 5 mmHg which elicited a bradycardia of 24 ± 12 and 42 ± 16 beats/min respectively. There were slight decreases in resting arterial pressure (from 66 ± 8 to 63 ± 8 mmHg) and heart rate (from 162 ± 12 to 157 ± 13 beats/min). In two experiments the intravenous dose of 5-HTP was increased to 4.5 and to 6.0 mg but no significant depression of noradrenaline-induced bradycardia was observed.

Discussion

Reflex bradycardia mediated solely through vagal activation was produced in spinal cats with intravenous pressor doses of noradrenaline. The 5-HT precursor, 5-HTP, administered into the fourth cerebral ventricle reduced this reflex bradycardia. Depression of the reflex responses occurred following 1.5 and 3.0 mg of 5-HTP and there was no appreciable difference in the duration or the magnitude of reduction of the bradycardia but the onset was faster with the higher dose. The action of 5-HTP can reliably be attributed to sites in the central nervous system and is not due to leakage into the peripheral circulation since intravenous injection of 3 to 6 mg of the drug had no appreciable effect on the vagal reflex bradycardia. In addition, Bogdanski, Weissbach & Udenfriend (1958) found that 5-HTP in intravenous doses of 60 mg/kg in dogs had no effect on noradrenaline-induced reflex bradycardia.

Central depression of reflex vagal activation by 5-HTP is mediated via conversion to 5-HT because inhibition of central L-amino acid decarboxylase with R04-4602 prevented the action of 5-HTP. In one dog that received a peripheral decarboxylase inhibitor, Antonaccio & Kervin (1977) observed that 5 mg/kg intravenous 5-HTP reduced intravenous noradrenaline-elicited reflex bradycardia. Our results show more directly that increased 5-HT levels in the brain following i.c.v. injection of the precursor, inhibit reflex vagal bradycardia in the cat. Inhibition of carotid occlusion pressor and tachycardia responses by a central action of 5-HT or its precursor are well documented (Bhargava & Tangri, 1959; McCubbin *et al.*, 1960; Dhawan *et al.*, 1967; Antonaccio & Robson, 1975; Tadepalli *et al.*, 1977). An earlier study in vagotomized cats showed that i.c.v. 5-HTP in total doses of 1.5 and 3.0 mg reduced the rise in sympathetic discharge elicited by carotid occlusion and decreased the sympathetically mediated reflex bradycardia (Tadepalli *et al.*, 1977). Together these studies show that elevation of brain 5-HT concentrations depresses the baroreceptor mediated reflex changes in sympathetic outflow as well as reflex vagal activation. In a recent paper (Tadepalli, Ho & Buckley, 1980), we showed that i.c.v. administration of the 5-HT receptor blocking agent, methysergide, enhances the vagal reflex bradycardia, suggesting the possibility that central 5-hydroxytryptaminergic mechanisms may be involved in the integration of baroreceptor-mediated reflex vagal activation.

Bugajski, Hano, Danek & Wantuch (1977) showed that intracerebroventricular 5-HT reduced gastric acid secretion in the rat suggesting a decrease in basal vagal activity. The decrease in resting heart rate in spinal cats following i.c.v. 5-HTP suggests an increase in basal vagal efferent activity. However, it was observed that the drug injected into 4CV can also enter the spinal cord as shown by the dye test used in each experiment. It was previously found that 5-HTP has a depressant effect on the discharge of preganglionic sympathetic neurones in the spinal cord of the cat (Naumayr, Hare & Frantz, 1974). The long latency of action of 5-HTP in reducing heart rate sug-

gests the possibility that at least part of the bradycardia observed with 5-HTP may be due to an action of the drug in the spinal cord. This possibility is now under investigation.

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