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Effects of nicotine on salivary amylase secretion from rabbit parotid gland

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Summary

1. The effects of nicotine on amylase secretion induced by auriculo-temporal nerve stimulation were studied.

2. Nicotine caused a transient increase in secretion as well as flow rate of amylase.

3. No difference in nicotine action was found between acute sympathetic decentralization of the gland and acute denervation.

4. The increase in amylase secretion due to nicotine was not inhibited by phenoxybenzamine, bretylium and chronic denervation, but was prevented by hexamethonium, propranolol and adrenalectomy.

5. The increase in flow rate due to nicotine was not inhibited by propranolol, chronic denervation and adrenalectomy, but was prevented by hexamethonium, phenoxybenzamine and bretylium.

6. These results show that the action of nicotine in increasing amylase secretion is neither a direct action on the ganglion nor on the nerve terminal of the cervical sympathetic nerve, but is an indirect action of catecholamines released from the adrenal medulla on the post-junctional receptors.

7. The study also suggests that the initial acceleration of salivary flow due to nicotine is characterized by a mechanism different from that of amylase secretion.

Introduction

The salivary glands are innervated by both sympathetic and parasympathetic nerves, receiving a main secretory innervation from the parasympathetic nervous system (Burgen & Emmelin, 1961). Amylase is a typical secretory digestive enzyme. Secretion of amylase from the rabbit parotid gland in response to parasympathetic stimulation is markedly increased by sympathetic stimulation and by administration of sympathetic amines (Yamamoto, Inoki, Tsujimoto & Kojima, 1968a). The β-adrenoceptors are the ones involved in this amylase secretory mechanism (Yamamoto, Inoki & Kojima, 1968b).

On the other hand, it has been reported that nicotine induces salivary secretion (Van Praag, 1855 ; Langley, 1918 ; Secker, 1934) and that a high concentration of nicotine persists longer in salivary glands than in other tissues (Hansson & Schmiterlöw, 1962 ; Yamamoto, Inoki & Iwatsubo, 1968c).

The present investigation was undertaken to clarify the effects of nicotine on amylase secretion from the rabbit parotid gland, with reference to the function of

the sympathetic nervous system, by determining amylase activity of saliva by single drop analysis.

Methods

Male rabbits weighing 2–3·5 kg were anaesthetized by intraperitoneal injection of urethane (1–1·5 g/kg) which has less effect on amylase secretion than other anaesthetics (Yamamoto, Inoki, Kojima, Ishida & Mizoguchi, 1966). Subsequent small doses of urethane were given in order to maintain the anaesthesia when necessary (usually at the onset). The trachea was exposed and cannulated. The parotid duct was cannulated using a short piece of stainless steel tubing which was connected to fine polyethylene tubing with a dead space of about 25 μl. The peripheral cut end of the auriculotemporal nerve, which is a postsynaptic parasympathetic nerve fibre, was electrically stimulated using bipolar platinum electrodes with submaximal shocks of 2 ms duration at 6–10 Hz. Voltage was usually adjusted to maintain a steady salivary flow in the range 100– 200 mg/minute. Each experiment was started after the flow rate was adjusted to the same level as in the first steady state. Each drop of saliva was collected separately to determine amylase activity and protein concentration (single drop analysis). In some experiments, unilateral sympathetic denervation was achieved by the removal of the superior cervical ganglion and sympathetic decentralization was accomplished by section of the preganglionic cervical sympathetic nerve on the opposite side. In some experiments, sympathetic chronically denervated preparations were made. Stimulation of the postganglionic cervical sympathetic nerve was applied using bipolar platinum electrodes with a square wave of 2 ms duration at 15 Hz and voltages were adjusted in order to cause dilatation of the pupil for 30 seconds.

Amylase activity was determined by modification of Bernfeld's method (Bernfeld, 1955 ; Yamamoto, Inoki, Kojima & Tamari, 1967) and protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). One unit of amylase activity was defined as the amount able to catalyse the formation of reducing sugar equivalent to 1 mg of maltose hydrate in 6 min at 30° C.

Drugs used were L-nicotine (nicotine), (−)-adrenaline hydrochloride (adrenaline), L-noradrenaline hydrochloride (noradrenaline), hexamethonium bitartrate (hexamethonium), phenoxybenzamine hydrochloride (phenoxybenzamine), propranolol hydrochloride (propranolol) and bretylium tosylate (bretylium). Phenoxybenzamine was injected slowly in geometrically increasing doses in the range 1·1, 2·3, 4·6 mg/kg to a total of 8 mg/kg at intervals of approximately 10 minutes. Bretylium was injected slowly for 3–5 minutes. Each interval of the injection of nicotine was 60–90 minutes. All drugs were administered intravenously via the femoral vein. Each result was obtained from five to ten rabbits and each figure shows an example of a typical experiment.

Results

Effects of nicotine on secretion and salivary flow induced by auriculotemporal nerve stimulation

Nicotine in doses of 30–2,000 μg/kg caused an increase in amylase activity and protein concentration. With the lowest dose these increases occurred without an

increase in salivary flow rate. Doses of nicotine greater than 100 μg/kg caused an increase followed by a decrease in flow rate. With the highest dose (2,000 μg/kg), salivary flow rate decreased so markedly that it was impossible to collect saliva. Typical and constant effects of nicotine on amylase and protein secretion and flow rate were obtained with a dose of 300 μg/kg. In all the experiments, the change in amylase activity of saliva was parallel to that in protein secretion (Fig. 1). In the following text these two parameters will be referred to jointly as amylase secretion.

Effects of acute sympathetic denervation and decentralization on amylase secretion caused by nicotine

The effects of nicotine on amylase secretion were observed in the sympathetic acutely denervated preparation and the acutely decentralized one in the same animal in order to examine the action of nicotine on the ganglion. Nicotine produced the same effect in both these preparations (Table 1).

Effects of hexamethonium, phenoxybenzamine, propranolol and bretylium on amylase secretion caused by nicotine

These effects are summarized in Table 1. Hexamethonium (2 mg/kg) caused a transient increase in flow rate and a decrease in concentration of amylase. Pretreatment with hexamethonium (2 mg/kg) 3 min before administration of nicotine (300 μg/kg) completely inhibited the increase in amylase secretion and salivary flow rate produced by nicotine.

After treatment with phenoxybenzamine alone (8 mg/kg), amylase secretion was increased. Phenoxybenzamine (8 mg/kg) given 30 min before nicotine did not inhibit the increase in amylase secretion produced by nicotine (300 μg/kg), but the initial acceleration in flow rate was inhibited.

Propranolol alone (300 μg/kg) produced a decrease in amylase secretion, but not in salivary flow rate. The increase in amylase secretion produced by nicotine was

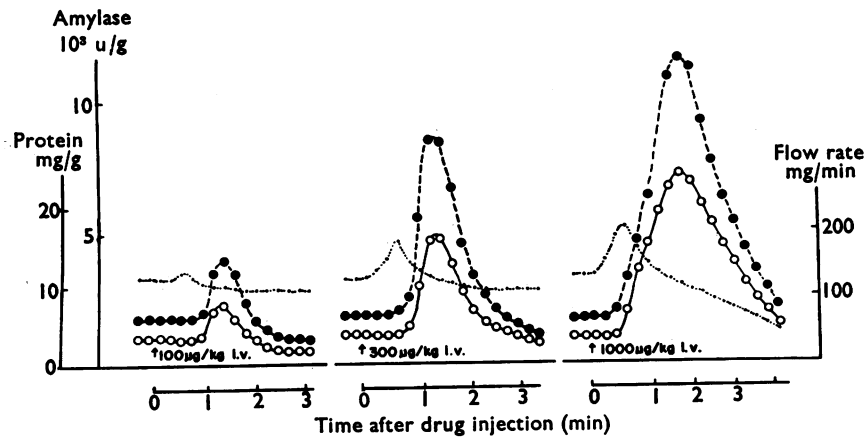

FIG. 1. Effects of nicotine in doses of 100, 300 and 1,000 μg/kg (intravenously) on amylase and protein secretion from rabbit parotid gland produced by auriculotemporal nerve stimulation in this and the following figures. Ordinate: amylase activity ($\times 10^3$ units/g) (—●—); protein concentration (mg/g) (—○—); and flow rate (mg/min) (- - - - -). Abscissa: time scale plotted arbitrarily so that there are equal intervals between drops.

markedly inhibited by pretreatment with propranolol (300 μg/kg) given 4 min before, but the initial acceleration in flow rate was scarcely inhibited.

Bretylium (10 mg/kg) acting alone slightly increased amylase secretion. Pretreatment with bretylium (10 mg/kg, 45 min before) had the following actions: the increase in amylase secretion produced by nicotine (300 μg/kg) was partially inhibited, that produced by electrical stimulation of the sympathetic nerve was completely inhibited (Fig. 2a) and that produced by noradrenaline was potentiated (Fig. 2b). The acceleration in flow rate due to nicotine and electrical stimulation was inhibited.

Effects of nicotine and adrenaline on the time course of amylase secretion

The initial acceleration in flow rate with nicotine in a dose of 300 μg/kg began about 15 s after the injection, while the increase in amylase and protein secretion began at 35–40 s, reaching a maximum by 70–80 seconds. The acceleration in flow rate with adrenaline followed a similar time course to the effect of nicotine. How-

TABLE 1. *Effect of drugs and surgical procedures on amylase secretion by auriculotemporal stimulation in rabbit parotid gland*

Treatment	Dose (μg/kg)	% Increase ±S.E.M. Flow rate	Amylase conc.	Protein conc.
Nicotine	300	150±43	286±37	227±23
Noradrenaline	3		440±71	386±63
Sympathetic stimulation		215±127	520±205	473±185
Hexamethonium	2,000		−47±6	−45±5
Hexamethonium	2,000*			
Nicotine	300	0·3±0·2	3±2	2±1
Phenoxybenzamine	8,000		75±19	82±45
Phenoxybenzamine	8,000†			
Nicotine	300	0·5±0·1	330±53	228±37
Propranolol	300		−79±18	−71±11
Propranolol	300‡			
Nicotine	300	113±30	5±4	4±2
Bretylium	10,000		39±20	31±15
Bretylium	10,000§			
Nicotine	300	0·4±0·1	180±31	138±9
Bretylium	10,000			
Noradrenaline	3		486±130	408±106
Bretylium	10,000			
Sympathetic stimulation		0·8±0·2	2±2	2±2
	Acute decentralization			
	100		175±26	150±8
Nicotine	300		282±36	275±43
	1,000		499±106	538±93
	Acute sympathetic denervation			
	100		150±8	159±21
Nicotine	300		275±43	249±51
	1,000		538±93	441±72
Adrenaline	3		487±25	302±25
	Sympathetic denervation—chronic			
			63¶±15	26¶±2
Nicotine	300	264±14	314±76	263±89
Adrenaline	3		512±21	412±17
	Adrenalectomy—60 min previously			
Nicotine	300	72±28	6±4	3±2

Increase in amylase and protein concentrations are expressed as percentage increase over the steady state (control) values. *, Given 3 min before the nicotine; †, given 4 min before; ‡, 30 min before; §, 45 min before. ¶, Compared with acute denervation.

ever, the increase in protein secretion with nicotine began 15–25 s later than that with adrenaline. The mean ± S.E.M.s of the time taken for the maximum responses to appear are shown in Table 2.

Effects of sympathetic denervation and adrenalectomy on amylase secretion caused by nicotine and adrenaline

Unilateral chronic sympathetic denervation 2 weeks before, and acute sympathetic denervation on the opposite side immediately before the experiment was

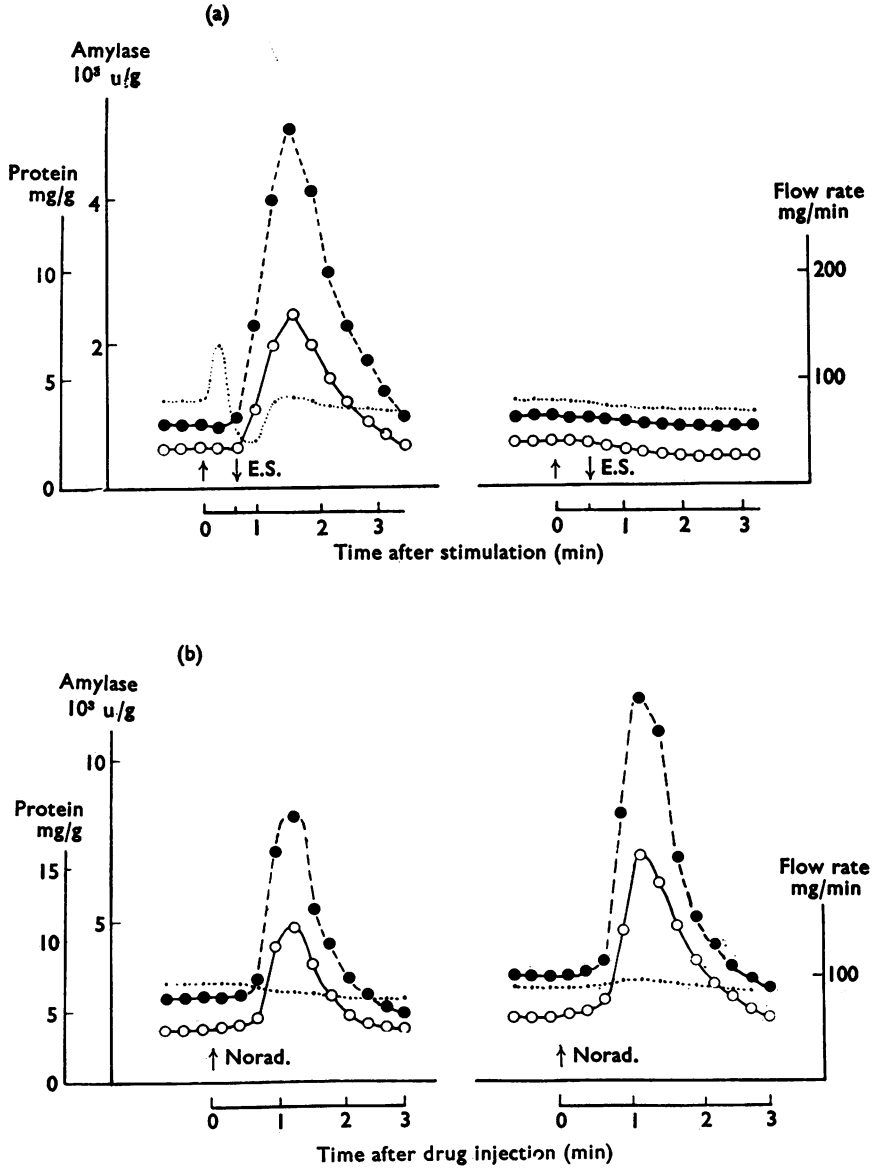

FIG. 2. Influence of sympathetic nerve stimulation (E.S., (a) left) and noradrenaline (3 μg/kg, (b) left) on basal amylase secretion. Effect of bretylium (10 mg/kg, 45 min previously) on these responses ((a) and (b) right).

TABLE 2. *Maximum response times (s) after the drug injection (mean ±S.E.M.)*

Drug administered	Flow rate	Amylase and protein secretion
Nicotine (200 μg/kg)	18±1·8	80±3·3
Adrenaline (3 μg/kg)	18±1·1	47±1·8

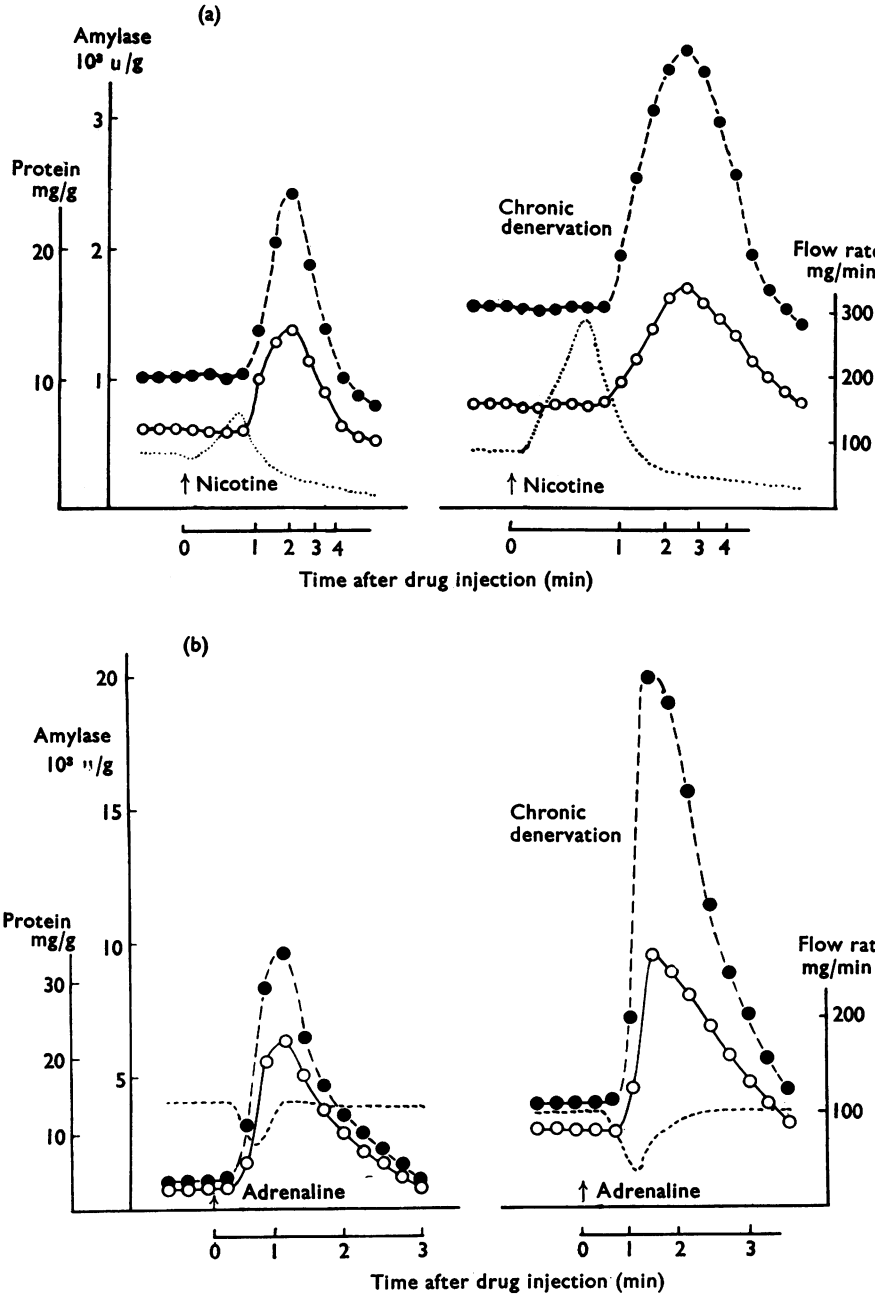

FIG. 3. Influence of nicotine (300 μg/kg (a) left) and adrenaline (3 μg/kg (b) left) on basal amylase secretion. Effect of chronic sympathetic denervation carried out 2 weeks previously on these responses ((a) and (b) right).

made in the same animal. In the chronically denervated gland, amylase secretion was higher than that in the acutely denervated gland (Table 1). The initial acceleration in salivary flow rate and the increase in amylase secretion produced by nicotine and adrenaline were augmented in the chronically denervated gland (Fig. 3a and Table 1).

Sixty minutes after the removal of the adrenal glands, the intravenous injection of 300 μg/kg of nicotine did not cause the increase in amylase secretion but the acceleration in flow rate was not abolished (Table 1).

Discussion

Secretion of amylase, a major digestive enzyme of saliva, is regulated both by the sympathetic and parasympathetic nervous systems (Schneyer & Hall, 1966 ; Yamamoto & Kojima, 1969) and the increase in amylase secretion is especially caused by sympathetic nerve stimulation, the β-adrenoceptors being involved in this mechanism (Kojima, 1967a). On the other hand, the initial acceleration in salivary flow produced by sympathetic stimulation is specifically antagonized by α-adrenoceptor blocking agents (Emmelin, Holmberg & Ohlin, 1965 ; Kojima, 1967a).

It was demonstrated by means of single drop analysis that there was a steady level in amylase activity in saliva due to auriculotemporal stimulation from rabbit parotid gland, and that accurate analysis of the actions of drugs on amylase secretion could be carried out during such a steady state (Kojima, 1967b).

The effects of nicotine on amylase and protein secretion, and salivary flow were observed in the steady state under the stimulation of auriculotemporal nerve. Intravenous injection of nicotine caused increases in amylase and protein secretion and also caused an initial acceleration in flow rate followed by a decrease. In all the experiments, the change in amylase secretion due to nicotine roughly paralleled the change in protein secretion as described in a previous report (Yamamoto *et al.*, 1968a). The increase in amylase secretion and salivary flow rate due to nicotine was inhibited by hexamethonium. No difference in nicotine effect was observed between the acutely denervated gland and the acutely decentralized one. This result showed that the effects of nicotine on amylase secretion and salivary flow rate were not due to an action of nicotine on the superior cervical sympathetic ganglion.

The increase in amylase secretion produced by nicotine was inhibited by propranolol, but not by phenoxybenzamine, whereas the initial acceleration in flow rate was inhibited by phenoxybenzamine, not by propranolol. As mentioned in the other reports (Kojima, 1967a ; Yamamoto *et al.*, 1968b), the receptors for catecholamines, isoprenaline, adrenaline and noradrenaline involved in amylase secretion were β-adrenoceptors and the initial acceleration in flow rate produced sympathetic stimulation was mediated through α-adrenoceptors. It can, therefore, be suggested that the effects of nicotine on amylase secretion and salivary flow are mediated through catecholamines released by nicotine.

The increase in amylase secretion produced by sympathetic nerve stimulation was completely inhibited after administration of bretylium, but that produced by nicotine was still present, though reduced. Bretylium depresses the effect of sympathetic nerve stimulation by inhibiting the release of noradrenaline in the nerve terminal, but not the release of adrenaline from the adrenal glands (Boura & Green, 1965), and responses of effector cells to circulating catecholamines may be

much increased. Bretylium showed so decisive an effect in this experiment that nicotine-induced amylase secretion is probably mediated through catecholamine released from adrenal glands.

In sympathetic chronically denervated glands, amylase activity in the steady state was much higher than that in acutely denervated glands and the amylase secretion became more sensitive to adrenaline in accordance with Cannon's law of denervation (Cannon, 1939). These results show that amylase secretion is under the control of the sympathetic nervous system. The increase in amylase secretion produced by nicotine was not inhibited by chronic denervation, but was somewhat augmented. It is considered that nicotine-induced amylase secretion is not mediated through catecholamine released from the sympathetic nerve terminals. The increase in amylase secretion with nicotine occurred 15–20 s later than that with the adrenaline. In hyperkalaemia and hyperglycaemia induced by nicotine, a similar result was obtained (Tsujimoto, Tanino & Kurogochi, 1965). Therefore, the delay of nicotine action on amylase secretion can be considered as the necessary time for a release of catecholamine from the adrenal glands. The removal of the adrenal glands abolished the effect of nicotine on amylase secretion.

From these results, it was concluded that the action of nicotine in increasing amylase secretion was neither a direct action on the ganglion nor an action on the nerve terminals of the cervical sympathetic nerve, but an indirect action on the post-junctional receptors of catecholamines released by nicotine from the adrenal medulla.

On the other hand, acceleration of the salivary flow produced by nicotine was inhibited by hexamethonium, phenoxybenzamine and bretylium. These drugs are supposed to inhibit nicotine-induced noradrenaline release from sympathetic nerve terminals (Burn, 1961 ; Bhagat, 1966). Emmelin has reported a mechanism of degeneration secretion (Emmelin, 1952), so the acceleration of the nicotine effect on flow rate and amylase secretion in the chronic sympathetic denervated gland may be attributed to a hypersensitivity at the receptors. It is not considered likely that the action produced by nicotine is due to an action on sympathetic nerve terminals. The time at which flow rate began to increase following nicotine and adrenaline was almost equal. After the removal of the adrenal glands, nicotine still caused an acceleration in flow rate.

These results suggest that the accelerating effect of nicotine on flow rate is not mediated through catecholamine released from adrenal glands. It can be considered that nicotine acts directly upon α-adrenoceptors as suggested by Burn, Leach, Rand & Thompson (1959) and Ferry (1963). The action of phenoxybenzamine on the flow rate can probably be explained as an inhibition of the direct action of nicotine, but the action of hexamethonium cannot be explained, and should be further investigated. Emmelin & Holmberg (1967) suggested that there was an anatomically unknown secretory nerve in dog parotid gland in addition to the auriculotemporal nerve and the cervical sympathetic nerve. The acceleration of the flow rate produced by nicotine may be due to stimulation of the ganglion of this unknown secretory nerve.

REFERENCES

BERNFELD, P. (1955). Amylase, α and β. In: *Methods in Enzymology*, ed. Colowick, S. P. & Kaplan, N. O., vol **1**, pp. 149–158. New York: Academic Press Inc.

BHAGAT, B. (1966). Response of isolated guinea-pig atria to various ganglion-stimulating agents. *J. Pharmac. exp. Ther.*, **154**, 264–270.

BOURA, A. L. A. & GREEN, A. F. (1965). Adrenergic neurone blocking agents. *Ann. Rev. Pharmac.*, **5**, 183–212.

BURGEN, A. S. V. & EMMELIN, N. G. (1961). *Physiology of the salivary glands.* London: Edward Arnold Ltd.

BURN, J. H., LEACH, E. H., RAND, M. J. & THOMPSON, J. W. (1959). Peripheral effects of nicotine and acetylcholine resembling those of sympathetic stimulation. *J. Physiol., Lond.*, **148**, 332–352.

BURN, J. H. (1961). A new view of adrenergic nerve fibres, explaining the action of reserpine, bretylium, and guanethidine. *Br. med. J.*, **1**, 1623–1627.

CANNON, W. B. (1939). A law of denervation. *Am. J. med. Sci.*, **198**, 737–750.

EMMELIN, N. (1952). Paralytic secretion of saliva. An example of supersensitivity after denervation. *Physiol. Rev.*, **32**, 21–46.

EMMELIN, N., HOLMBERG, J. & OHLIN, P. (1965). Receptors for catecholamines in the submaxillary glands of rats. *Br. J. Pharmac. Chemother.*, **25**, 134–138.

EMMELIN, N. & HOLMBERG, J. (1967). Impulse frequency in secretory nerves of salivary glands. *J. Physiol., Lond.*, **191**, 205–214.

FERRY, C. B. (1963). The sympathomimetic effect of acetylcholine on the spleen of the cat. *J. Physiol., Lond.*, **167**, 487–504.

HANSSON, E. & SCHMITERLÖW, C. G. (1962). Physiological disposition and fate of C^{14}-labelled nicotine in mice and rats. *J. Pharmac. exp. Ther.*, **137**, 91–102.

KOJIMA, S. (1967a). Pharmacological studies on amylase secretion from rabbit parotid gland. 2. On adrenergic receptors in amylase secretion. *Folia pharmac. jap.*, **63**, 169–178.

KOJIMA, S. (1967b). Pharmacological studies on amylase secretion from rabbit parotid gland. 1. A role of sympathetic nervous system on amylase secretion induced by parasympathetic nerve stimulation. *Folia pharmac. jap.*, **63**, 161–198.

LANGLEY, J. N. (1918). On the stimulation and paralysis of nerve cells and nerve endings. Part II. Paralysis by curari, strychnine and brucine and its antagonism by nicotine. *J. Physiol., Lond.* **52**, 247–266.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

SCHNEYER, C. A. & HALL, H. D. (1966). Autonomic pathways involved in a sympathetic-like action of pilocarpine on salivary composition. *Proc. Soc. exp. Biol. Med.*, **121**, 96–100.

SECKER, J. (1934). The humoral control of the secretion by the submaxillary gland of the cat following sympathetic stimulation. *J. Physiol., Lond.*, **82**, 293–304.

TSUJIMOTO, A., TANINO, S. & KUROGOCHI, Y. (1965). Effect of nicotine on serum potassium and blood glucose. *Jap. J. Pharmac.*, **15**, 415–422.

VAN PRAAG, J. L. (1855). Nicotin. Toxikologisch-pharmakodynamische Studien. *Virchows Arch. path. Anat. Physiol.*, **8**, 56–102.

YAMAMOTO, I., INOKI, R., KOJIMA, S., ISHIDA, H. & MIZOGUCHI, K. (1966). Effects of some general anesthetics on the salivary secretion. *Folia pharmac. japon.*, **62**, 213–219.

YAMAMOTO, I., INOKI, R., KOJIMA, S. & TAMARI, Y. (1967). Studies on salivary secretion induced by electric stimulation of auriculotemporal nerve in special reference to salivary flow and amylase activity. *Folia pharmac. jap.*, **63**, 153–160.

YAMAMOTO, I., INOKI, R., TSUJIMOTO, A. & KOJIMA, S. (1968a). The role of the sympathetic nervous system in amylase secretion elicited by parasympathetic nerve stimulation. *Eur. J. Pharmac.*, **3**, 117–122.

YAMAMOTO, I., INOKI, R. & KOJIMA, S. (1968b). Adrenergic receptors in amylase secretion from rabbit parotid gland. *Eur. J. Pharmac.*, **3**, 123–130.

YAMAMOTO, I., INOKI, R. & IWATSUBO, K. (1968c). Penetration of nicotine-^{14}C into several rat tissues *in vivo* and *in vitro*. *Toxic. appl. Pharmac.*, **12**, 560–567.

YAMAMOTO, I. & KOJIMA, S. (1969). Salivary amylase secretion from parotid gland and adrenergic receptors. *Igaku No Ayumi.*, **68**, 347–351.

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