Effects of adenosine and its derivatives on anaphylaxis in vitro of smooth muscle from guinea-pig uterus

Compounds tested	No. animals	Histamine equivalent[a] of anaphylactic response in the presence of the compound tested	Hitamine equivalent[a] of control response	P	Influence (%)
Cyclic AMP[b]	7	10.2 ± 9.99[d]	9.47 ± 10.8[d]	>0.7	
Adenosine[b]	8	20.7 ± 14.9	9.62 ± 9.60	<0.05	+115
AMP[b]	9	4.74 ± 3.78	2.25 ± 1.83	<0.05	+111
Dibutyryl cyclic AMP[c]	9	1.35 ± 1.06	2.44 ± 2.14	<0.05	−45

[a] μg of histamine dihydrochloride added in 20 ml tissue bath. [b] 4.65 μ*M*. [c] 23.3 μ*M*. [d] Mean ± standard deviation.

dence supporting this hypothesis was obtained in the case of human leucocytes[3].

In the present experiments, the effect of cyclic AMP on anaphylaxis of smooth muscle was studied in vitro. Adenosine and adenosine-5′-monophosphate (AMP) also were studied, since the 6-amino group which is common to the purine nucleus of these compounds has been suggested to show a pharmacological activity on smooth muscles[4].

Materials and methods. Most of the procedures for the present study have been described in a previous paper[4]. Smooth muscle pieces for the in vitro anaphylaxis were obtained from the uterine horns of virgin guinea-pigs, and passive sensitization of the muscle pieces was made with anti-bovine serum albumin rabbit antiserum. For the study of the effects of AMP and dibutyryl cyclic AMP, a pool of antiserum which was different from the pooled serum for the initial series of experiments on adenosine and cyclic AMP was used. Concentration of the test compounds was 4.65 μ*M* in the tissue bath, only dibutyryl cyclic AMP being tested in a higher concentration of 23.3 μ*M*. The size of each reaction was expressed in the histamine equivalent of isotonic smooth muscle contraction obtained from the dose-response curve by histamine dihydrochloride on the individual muscle piece. From 1 animal, 4 smooth muscle pieces were used for an experiment: Averages of duplicate determinations with 2 pieces for control and 2 others for test, respectively, were calculated. Adenosine and AMP were obtained from Wako Pure Chemical Industries Ltd., Osaka (Japan); Cyclic AMPs were supplied by Sigma Chemical Co., St. Louis (USA).

Results and discussions. As shown in the Table, cyclic AMP, in a concentration of 4.65 μ*M*, failed to show an effect on the anaphylactic reaction. However, in the form of dibutyryl derivative which enters the cell more readily, and in a higher concentration, cyclic AMP inhibited the anaphylactic reaction. This inhibitory action may be attributed to the direct relaxing effect of cyclic AMP on smooth muscle and/or possible inhibition of histamine release by the compound, and is in favour of the hypothesis that the inhibitory actions on anaphylaxis of catecholamines and xanthines include the cyclic AMP system.

In contrast to cyclic AMP, adenosine and AMP in the concentration of 4.65 μ*M* enhanced the anaphylactic reaction. Thus, the marked intensification of anaphylactic reaction of smooth muscle by the same molar concentration of adenosine-5′-diphosphate (ADP) and adenosine-5′-triphosphate (ATP) observed in the previous study[4] seems to be related significantly to the pharmacological property inherent to the adenosine and its derivatives, cyclic AMP being the exception[5].

Zusammenfassung. Zyklisches 3′, 5′-Adenosinmonophosphat hindert in vitro die Anaphylaxis des glatten Muskels vom Meerschweinchenuterus, während Adenosin und Adenosin-5′-Monophosphat die anaphylaktische Reaktion erhöhen.

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Catalepsy Produced by Intraventricular Injection of Nicotine[1]

Catalepsy in rats[2], mice[2] and dogs[3,4] by subcutaneous or intravenous injections of nicotine has scarcely been studied. On the other hand, catalepsy by intraventricular injection of this drug in the conscious cat has not yet been studied. It was therefore of interest to know whether nicotine can produce catalepsy in conscious animals after intraventricular administration acting directly on the central nervous system. Furthermore, with the method of intraventricular application the brain structures close to or directly in contact with the ventricular surface can be implicated in the appearance of catalepsy.

Six cats of both sexes, weighing from 2.0 to 2.6 kg, were used in these experiments. For the injection of drugs into the cerebral ventricles a Collison cannulae was implanted aseptically into the left lateral ventricle during pentobar-

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biton sodium (35–45 mg/kg) anaesthesia, as described by FELDBERG and SHERWOOD[5]. Penicillin was administered postoperatively and a 3–5-day interval elapsed before the first experiment. All solutions were injected slowly under aseptic conditions in volumes of 0.2 ml and washed in with 0.1 ml of 0.9% NaCl. The cats were observed continously for 2–6 h and intermittently for 24 to 48 h. The drugs used were nicotine hydrogen tartrate and tetraethylammonium chloride.

The most striking effect of nicotine after its intraventricular injection in doses from 1.0 to 4.0 mg was a condition of catalepsy. The signs of catatonia appeared within 10 min and persisted for about 40 min. When fully developed the cat could be placed in nearly erect position with forepaws over the rung of an inverted stool. The cat remained in this position for about 15 sec. Thereafter the cat slowly climbed down. Moreover, when the cat was induced to walk, it ceased moving after a few steps and stood motionless for a few minutes. Finally, it lay down on its belly. During this time the eyes were half open and the cat showed little interest in its surroundings with no signs of affection. When the stage of catalepsy wore off, no spontaneous movements were observed and the cat would sit usually under a bench motionless for hours if undisturbed. The sedation and stupor lasted up to 12 h. It is interesting to note that in some experiments catalepsy developed after convulsions.

Intraventricular administration of tetraethylammonium in dose of 2 mg potentiated the signs of catalepsy. When the intraventricular injection of tetraethylammonium was preceded by an intraventricular administration of nicotine, the cat could be placed in nearly erect position with its forepaws over the rung of an inverted stool for about 90 min.

Apart from the signs of catalepsy an intraventricular administration of nicotine in doses from 1.0 to 4.0 mg produced mydriasis, salivation, piloerection, vomiting, ataxia, tremor, respiratory embarassements, rigidity, convulsions and sometimes akathisia. Control injections of 0.4 ml of 0.9% saline caused no visible changes.

The present experiments show that nicotine produced catalepsy in conscious cats when injected into the cerebral ventricles. Intraventricular application of bulbocapnine[6], anticholinesterase[7], morphine[8] and prostaglandin[9] in unanaesthetized cats and rabbits also produced catalepsy. Furthermore, experimental syndrome of catatonia can be obtained by placing electrolytic lesions in the upper brain stem[10]. The lesions of the border between upper tegmentum and the posterior hypothalamus are known to produce catatonic syndrome[11]. These regions might be reached by intraventricular injection of nicotine from the third ventricle. Nicotine in high doses is known to interrupt nervous pathways by producing a block of synaptic transmission in autonomic ganglia or in neuromuscular junction. By analogy, it can be supposed that nicotine in high doses injected intraventricularly produced a kind of pharmacological lesion paralyzing nerve cells and interrupting probably some specific pathways, causing catalepsy.

Résumé. La nicotine, aux doses de 1.0 à 4.0 mg, injectée par voie intraventriculaire à des chats non-anesthésiés, provoque la catalepsie, la sédation et la stupeur. Les signes de catalepsie apparaissent d'ordinaire 10 min après l'injection et disparaissent au bout de 40 min. L'application intraventriculaire de tétra-éthyl-ammonium potentialise les symptômes de catalepsie dûs à la nicotine.

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Development of Behavioural Tolerance to Nicotine in the Rat[1]

Although chronic self-administration of nicotine (as a constituent of tobacco) is widespread, most studies of the behavioural effects of this drug on experimental animals have been concerned with the consequences of acute administration. Vertical rearing activity in rats has been shown to be susceptible to the acute effects of nicotine in a number of studies[2–5]. This component of the general activity of the rat is also useful in the assessment of the effects of repeated administration of drugs since it is known to stabilize when animals are repeatedly exposed to the same test situation[6], thereby providing a constant baseline against which drug effects can be assessed.

Repeated administration of a drug often results in a diminished effect due to the development of tolerance[7]. It has also been found[8,9], that when drugs are given repeatedly before animals are exposed to a behavioural test, drug withdrawal can have behavioural consequences even though physiological dependence may not be apparent.

There is evidence that drug effects on behaviour may be greater when the drugs are administered before exposure to completely novel, as against familiar, situations[10–12]. This effect could be confused with the development of tolerance in experiments in which drug administration is repeatedly followed by exposure to some test situation. In order to minimise this possibility the present study was so designed that animals had already received several exposures to the experimental situation before drug administration was commenced.

Materials and method. 16 Roman control (RCA) strain rats, aged 180 days, were used in the study. All the rats were males.

The apparatus used to measure rearing activity consisted of a transparent acrylic plastic tube 63 cm high with an internal diameter of 23 cm. A stainless steel band, 1.3 cm wide, attached to the outer circumference of the tube 25 cm from the base, functioned as the probe of a proximity meter (capacitance transducer). Vertical movement of the rat resulted in a capacitance change proportional to the distance between the animal's head and the probe. This capacitance change caused the proximity meter to produce a varying voltage output which was then recorded as an analogue print-out curve on a moving-pen recorder.