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Research Papers

Effects of tyramine on a spinal reflex in the anaesthetised chick

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The effects of tyramine on polysynaptic spinal reflexes in anaesthetised chicks and cats have been studied. Very large intravenous doses of tyramine depressed the reflexly evoked contractions of skeletal muscles by an action exerted in the spinal cord. Since tyramine is normally present in the central nervous system, its function may be to exert some inhibitory controlling influence on muscular activity.

THE presence, in mammalian tissues, of an enzyme capable of catalysing the decarboxylation of tyrosine to tyramine was demonstrated by Lovenberg, Weissbach & Udenfriend (1962); tyramine is normally present in the urine of man, the amount excreted being about 0·2–0·5 mg/day (Levine, Oates, Vendsala & Sjoerdsma, 1962). By a combination of solvent extraction and fluorimetric assay, Spector, Melmon, Lovenberg & Sjoerdsma (1963) examined mammalian tissues for the presence of tyramine. They concluded that the amine is present in amounts of 1 to 6 μg/g in various parts of the central nervous system of rats, rabbits, dogs and cats, the highest concentrations being in the brain stem and spinal cord. Spector and others also mention some experiments in which they found that after convulsive doses of strychnine, the tyramine level in the spinal cord of rabbits was decreased.

These results suggest that tyramine may, either directly or indirectly, influence the motoneurones innervating skeletal muscles. However, in preliminary experiments on the cat, intravenously injected tyramine, in doses up to 2 mg/kg, was found to be without effect on spinal reflexes.

It was considered that the inactivity of intravenous tyramine in the mammal might be because it cannot penetrate from the blood stream to its possible site of action in the central nervous system. Studies by Zaimis (1960) and Key & Marley (1962) have suggested that the blood-brain barrier, at least with respect to some drugs, is defective in the domestic fowl during the first few days after hatching and for this reason it was decided to study the effects of tyramine on a spinal reflex evoked in young chicks.

Methods

The experiments were made on 84 male chicks (Silver Link) ranging in age from 1 to 15 days after hatching. The chicks were anaesthetised with chloralose (60 mg/kg) injected intraperitoneally. The chick was laid on its back, the trachea cannulated and artificial respiration commenced immediately. A stout pin was placed through the lower end of the femur of the right leg and firmly clamped so that the lower leg could

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swing freely. One end of a thread was tied to the ankle and the other end was attached, via a pulley-wheel, to a light spring-loaded writing lever. Extension of the limb caused the lever to write on a kymograph. Crossed extensor reflexes were elicited once every 15 or 20 sec by stimulation of the peripheral end of the central stump of the severed contralateral sciatic nerve with single rectangular shocks of 0·5–1 msec duration. Injections were made, in a volume not exceeding 0·2 ml, through a fine polythene cannula tied into a jugular vein. In many experiments, the sciatic artery of the left leg was also cannulated and arterial blood pressure was recorded by means of a Condon manometer. Twelve chicks were treated with 1 mg/kg of reserpine injected subcutaneously on each of the first and second days after hatching. Reflex contractions

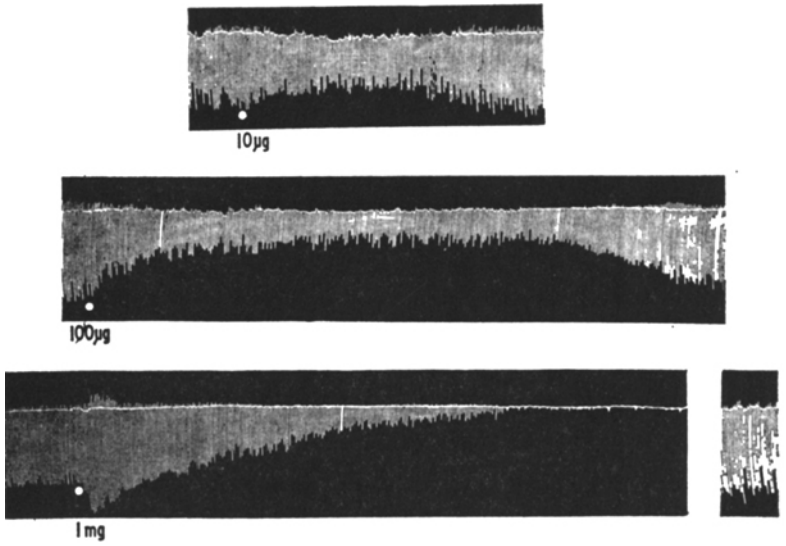

FIG. 1. Chick aged 2 days and weighing 32 g. Crossed extensor reflex elicited every 15 sec; contractions downwards. At the white dots tyramine was injected intravenously in the absolute doses indicated. The second part of the lowest record was recorded 20 min after the end of the first part.

were recorded on the third day. Twenty-four chicks, aged between 1 and 6 days, were decapitated during the experiment. The head was severed after tightly ligating the neck excluding the trachea and the cannulated jugular vein. Ten chicks were spinalised during the experiment by sectioning the cord at the level of C 10 between two ligatures tied tightly round the spinal column and adhering muscles.

In some experiments contractions of the gastrocnemius muscle were recorded in response to motor nerve stimulation. In these experiments, a pin was placed through the lower end of the femur and another through the lower end of the tibia, so that the lower limb could be clamped in a horizontal position. The tendon of the gastrocnemius muscle was attached, via a pulley wheel, to the writing lever and maximal twitches

were elicited by stimulation of the peripheral stump of the severed ipsilateral sciatic nerve with single rectangular shocks of 100 μsec duration.

Similar experiments were made on three cats under chloralose anaesthesia (80 mg/kg injected intravenously). The method was identical to that previously described (Bowman & Sanghvi, 1963). Flexor reflex contractions of a tibialis anterior muscle and crossed-extensor reflexes of a quadriceps femoris muscle were recorded. The reflex contractions of the tibialis anterior muscle were elicited by stimulation of the ipsilateral musculo-cutaneous branch of the peroneal nerve with single rectangular shocks of 0·5 msec duration. The reflex contractions of the quadriceps muscle were elicited as in chicks but with 0·5 sec bursts of repetitive stimulation at a frequency of 10/sec. Drugs were injected intravenously through a cannula in a jugular vein and arterial blood pressure was recorded from a common carotid artery.

The drugs used were tyramine hydrochloride (BDH), 3-hydroxytyramine hydrochloride (dopamine, BDH), noradrenaline bitartrate (BDH), strychnine nitrate (BDH), reserpine (Serpasil ampoules, Ciba) and nialamide (Pfizer).

Results

Intravenously injected tyramine always depressed the crossed extensor reflex in chicks but the effective doses and the duration of the depression varied in different experiments. Fig. 1 illustrates an experiment on a 2 days-old chick in which tyramine was unusually effective. A dose of 10 μg produced a definite depression of the contractions and a dose of 1 mg completely abolished them after an initial small potentiation. In this experiment the time from injection to maximal depression was longest with the largest dose possibly because, with the brain intact, the effect of large doses of tyramine was the result of both a stimulant and a depressant action in the central nervous system.

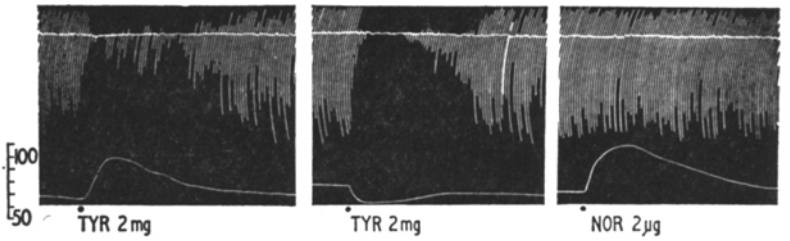

FIG. 2. Chick aged 2 days and weighing 35 g. Crossed extensor reflex (upper record) recorded as in Fig. 1. Blood pressure (lower record) recorded from the sciatic artery (calibration in mm Hg). At TYR, tyramine and at NOR, noradrenaline injected intravenously in the absolute doses indicated. The first two panels show the first and the fourth injection of the same dose of tyramine.

Fig. 2 illustrates an experiment, again on a 2 days-old chick, in which the effective doses of tyramine lay at the other extreme of the dose-range. In this experiment an intravenous dose of 2 mg of tyramine produced

an immediate but relatively short-lasting depression of the contractions. In most experiments, including all those in which chicks more than 6 days old were used, large doses of tyramine of about 0·5 mg/10 g body weight were required to depress the reflex contractions. The depression was occasionally preceded by a brief stimulant effect. Even the largest doses required were without effect on maximal twitches of the gastrocnemius muscle elicited by motor nerve stimulation.

At the start of the experiment, the blood pressure of the anaesthetised chick was usually between 70 and 100 mm Hg. During the first hour or so, the blood pressure usually fell gradually, occasionally to as little as 40 mm Hg. However, the amplitude of the reflex contractions was little effected by this fall in blood pressure. Large doses of tyramine (0·2–0·5 mg/10 g body weight) produced rises in blood pressure of some 30 to 60 mm Hg (Figs 2 and 5) which were often preceded by a small fall (Fig. 5). Tachyphylaxis of the pressor effect was usually quickly established when large doses were repeatedly injected. The first panel of Fig. 2 illustrates the effect of an initial large dose of tyramine. The second panel of Fig. 2 illustrates the effect of the fourth injection of the same dose. In contrast to the first injection the fourth injection produced a fall in blood pressure but a similar depression of the reflex contractions was produced. The third panel of Fig. 2 illustrates the absence of effect on the reflex contractions of a dose of noradrenaline which produced a rise in blood pressure greater than that produced by the first injection of tyramine.

In chicks pre-treated with reserpine, the pressor effect of tyramine was weak or absent but a depression of the reflex contractions similar to that occurring in the non-reserpinised chicks was produced.

Decapitation of the chick caused a rise in blood pressure lasting 1–2 min and this was followed by a fall to 30–40 mm Hg where it remained constant, in the absence of drugs, for the remainder of the experiment (Fig. 5). Decapitation always abolished the reflex contractions but this effect was not always immediate, suggesting that it was not simply the result of spinal shock. Fig. 5 illustrates an experiment in which the reflex contractions persisted unchanged for 5–6 min after decapitation but disappeared as the blood pressure fell to 30 mm. However, in other experiments, in which the blood pressure was already low and was not lowered further by decapitation, the reflex contractions disappeared immediately on severing the head. It, therefore, appeared that the abolition of reflex contractions was the combined result of mild spinal shock, produced by decapitation, and of the fall in blood pressure. When left alone, reflex contractions did not re-appear during the rest of the experiment (up to 3 hr after decapitation). However, contractions could always be restored at any time after decapitation by the intravenous injection of small doses of strychnine (1–2 μg/chick). After the initial large potentiation produced by strychnine, the amplitude of the contractions subsided to a lower level where they remained constant for the rest of the experiment. In the experiment of Fig. 5, the contractions remained constant, at the level illustrated at the end of the lower panel,

for a further 2 hr, after which time the experiment was terminated. Tyramine, injected at any time after decapitation, produced a depression of the restored reflex contractions similar to that occurring before decapitation.

When spinalisation was done by sectioning the cord in the cervical region, the fall in blood pressure was less severe than that following decapitation. Reflex contractions were depressed in amplitude but not

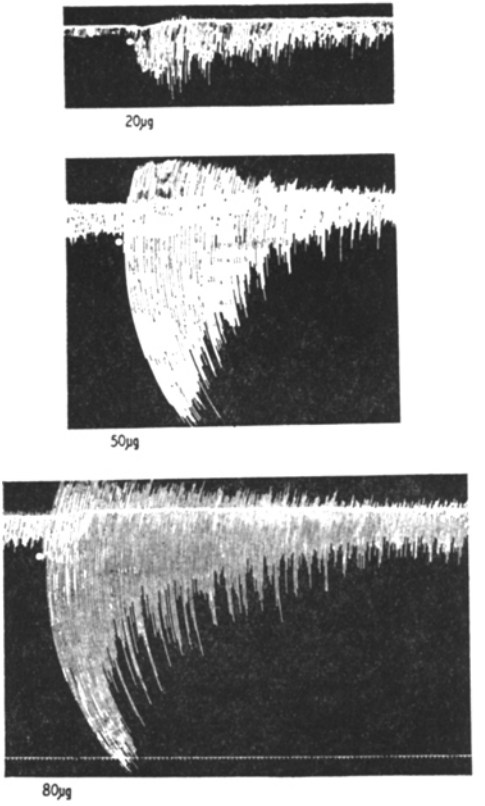

FIG. 3. Chick aged 15 days and weighing 124 g. Recording as in Fig. 1. At the white dots, strychnine injected intravenously in the absolute doses indicated. Time trace on the lowest panel, 0·5 min intervals.

abolished by this procedure, and, in the absence of drugs, they continued at a constant level for the remainder of the experiment. Contractions elicited from these preparations were always more constant in amplitude than those produced when the central nervous system was left intact. Tyramine injected after spinalisation produced a depression of the reflex contractions similar to that occurring before transection of the cord.

Tyramine was also tested for an anti-strychnine action. Fig. 3 illustrates the potentiating effect of three doses of strychnine on the reflex contractions elicited in a 15 days-old chick. On a dose per body weight basis,

strychnine was found to be more active, the younger the chick. In fact the effective doses of strychnine in μg was found to correspond approximately to the age of the chick in days. Thus in a 1 day-old chick, the smallest effective dose of strychnine was about 1–2 μg intravenously while in a 15 days-old chick, 15–20 μg was required. The gain in weight of the chicks over the first 15 days was only 300–500%.

The effect of tyramine on the strychnine potentiation depended on the dose of strychnine and on the time of injection of tyramine. Fig. 4

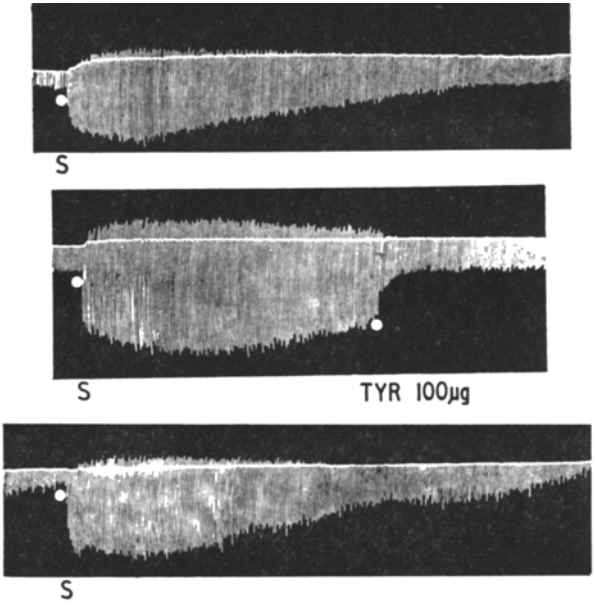

FIG. 4. Chick aged 2 days and weighing 30 g. Recording as in Fig. 1. At S, 2 μg of strychnine and at TYR, 100 μg of tyramine injected intravenously. 35 min elapsed between injections of strychnine.

illustrates an experiment on an intact chick in which 100 μg tyramine, injected towards the end of the potentiation produced by a small dose of strychnine, depressed the contractions to the pre-strychnine level. When the dose of strychnine was larger and when tyramine was injected soon after strychnine, no depressant effect was produced even by doses of tyramine which, before strychnine, were big enough to abolish the reflex contractions completely (Fig. 5, upper panels). When tyramine was injected at the height of the strychnine potentiation in an intact chick, the evoked contractions were often slightly increased rather than depressed (Fig. 5, upper panels) and sometimes rapid additional contractions of the muscle were produced. When tyramine was injected before strychnine, the potentiating effect of strychnine was only slightly depressed.

In the decapitate preparation and the preparation spinalised in the cervical region, tyramine always blocked the reflex contractions in the

presence of strychnine irrespective of the time it was injected. Under these circumstances, the depression of contractions was always preceded by rapid and powerful contractions of the muscle which were not related to the electrical stimulation. Fig. 5 illustrates an experiment in which, in the intact preparation, tyramine depressed the normal reflex contractions but did not depress potentiation produced by strychnine. However, after decapitation, the same dose of tyramine abolished the effect of strychnine after an initial short-lasting stimulant effect.

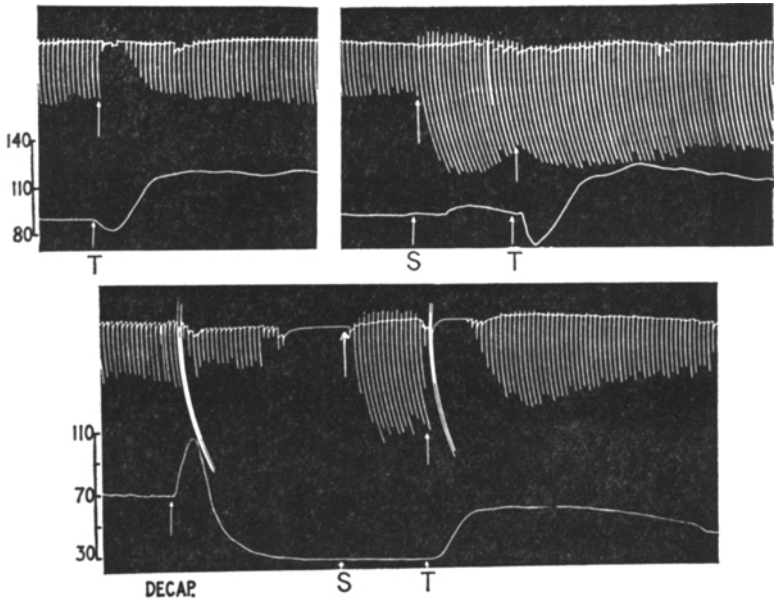

FIG. 5. Chick aged 4 days and weighing 63 g. Recording as in Fig. 2. Crossed-extensor reflexes elicited once every 20 sec. At T, 1 mg tyramine and at S, 5 μg strychnine injected intravenously. At DECAP, the head was completely severed.

In a few spinalised preparations, the effect of dopamine was tested. In similar doses, dopamine produced greater pressor effects than tyramine but it did not depress the reflex contractions either in the presence or in the absence of strychnine. When the blood pressure of the chick was very low, the pressor effect of dopamine was often accompanied by a small increase in the amplitude of the contractions.

The depressant action of tyramine, both in the presence and in the absence of strychnine, was potentiated by the previous injection of the monoamine oxidase inhibitor, nialamide (100 μg/10 g weight). After nialamide, the depressant effect of tyramine (0·25 mg/10 g weight) was occasionally permanent, no recovery of the reflex contractions occurring throughout the remainder of the experiment.

The doses of tyramine required to affect the reflex contractions in the chick were extremely large. For example, doses of 1–2 mg in a 2 days-old

chick weighing about 30 g are equivalent, on a body weight basis, to doses of about 100–200 mg in an average sized cat. Since the chick could withstand repeated doses of this magnitude, it was decided to test the effect of equivalent doses in cats.

In the cat, intravenous doses of about 50 mg/kg caused a depression of both the crossed extensor and the flexor reflex contractions from which recovery was slow and incomplete. Fig. 6 illustrates this effect on the flexor reflex and shows that the depression was preceded by a powerful but short-lasting stimulant effect. Although the evoked reflex contractions were completely abolished, contractions of the muscle, unrelated to the electrical stimulation, occurred intermittently after the injection of tyramine. At the height of the block of the flexor reflex, stimulation of the motor nerve still evoked maximal twitches of the tibialis anterior muscle. A stimulant action of large doses of tyramine was also evident in what appeared to be an analeptic effect. Immediately after injection, respiratory rate and depth were increased and side to side head movements occurred. This effect lasted for about 5 min after injection.

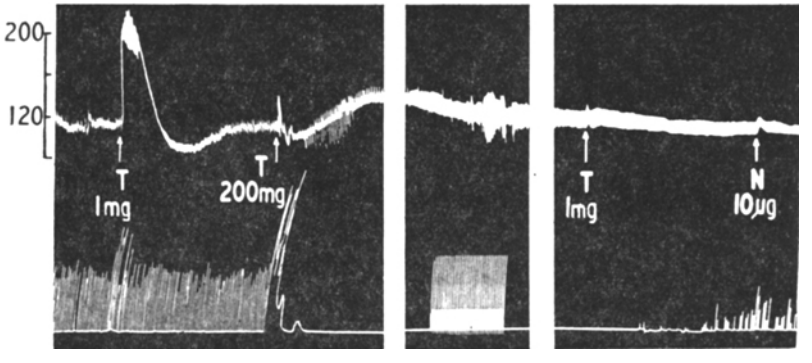

FIG. 6. Adult cat weighing 4 kg. Flexor reflex contractions of a tibialis anterior muscle elicited once every 10 sec (contractions upwards) and blood pressure from a carotid artery (calibration in mm Hg). At T, tyramine and at N, noradrenaline injected intravenously in the absolute doses shown. The middle panel shows maximal twitches of the tibialis anterior muscle elicited by stimulation of the sciatic nerve once every 10 sec at a time when the reflex contractions were completely abolished. The gaps between panels represent intervals of about 15 min. The recovery of the reflex contractions shown at the end of the third panel was the maximal degree of recovery which occurred.

The effects of large doses of tyramine on the blood pressure of the cat were surprisingly small. An intravenous injection of 50 mg/kg caused a small and transient rise after which the blood pressure quickly returned to normal. The effect of 50 mg/kg was actually smaller than the effect of 0·25 mg/kg given previously (Fig. 6). After the large dose of tyramine, subsequent doses of the same drug, and of noradrenaline, were completely without effect on the blood pressure (Fig. 6).

The effect of tyramine in the cat was therefore similar to its effect in the chick. However, the cat was less able than the chick to withstand repeated large doses of tyramine. In two of the three experiments on cats, a second large dose of tyramine caused a profound fall in blood pressure, cessation of respiration and death.

Discussion

The depressant effect of tyramine on the polysynaptic reflexes was most probably exerted in the central nervous system since the amine did not depress twitches of skeletal muscle elicited by direct motor nerve stimulation. Since the same effect was produced in the spinalised preparations, the site of action may be located in the spinal cord. The effect did not appear to be a consequence of cardiovascular changes for the following reasons: (1) it still occurred when complete tachyphylaxis to the cardiovascular effects had been established; (2) doses of noradrenaline or dopamine which produced equal or greater rises in blood pressure did not depress the reflex contractions; (3) in the reserpinised chick, tyramine produced very little change in blood pressure but still depressed the reflex contractions. Spectrophotofluorimetric assays made in this laboratory by Callingham, Cass & Osuide of the brains and hearts of chicks treated with the same doses of reserpine have shown a 50 to 75% depletion of catecholamine content. This suggests that, unlike its peripheral actions (Burn & Rand, 1958), the central actions of tyramine are not mediated through noradrenaline release.

The central effects of tyramine were produced in the adult cat by doses equivalent in size to those found effective in the chick. In both species the effective doses were very large suggesting that the particular barrier to the site of action of tyramine is as well developed in the chick as it is in the cat. This does not contradict the general statement that the blood-brain barrier in the chick is under-developed, since it is clear from the results of Zaimis (1960) and Key & Marley (1962) that sympathomimetic amines do penetrate well into some areas of the central nervous system of the chick.

Since tyramine has been found in the central nervous system, and since it exerts a depressant action on polysynaptic reflex activity, its function may be to exert an inhibitory modulating influence on synaptic activity. Although the intravenous doses of tyramine required were large, the amine might prove very active if it could be applied directly to the site of the depressant action. We have not yet made any real attempt to analyse the site and mechanism of action of tyramine. However, it did not appear to inhibit the anterior horn cells directly since, at the height of the block of the evoked contractions, tyramine occasionally initiated irregular contractions of the muscle which were unrelated to the electrical stimulation.

The depressant action of tyramine was antagonised by strychnine. The convulsive action of strychnine is due to interruption of transmission at inhibitory synapses, probably by competitive blockade of the inhibitory

transmitter (Eccles, Fatt & Koketsu, 1954; Curtis, 1959, 1962; Eccles, 1964). However, since tyramine bears little chemical relationship to strychnine, it seems unlikely that both should act on the same receptor. Furthermore, the finding of Spector & others (1963), that the tyramine content of the central nervous system is depleted by strychnine, is difficult to reconcile with the possibility that tyramine is released from the nerve endings at inhibitory synapses. It may be, however, that when inhibitory transmitter is blocked by strychnine, the unchecked excitation activates some mechanism which causes the release of tyramine. The tyramine may then be exposed to destruction by amine oxidase and its concentration in the CNS depleted. It might be that tyramine acts, not by a direct depressant action, but by stimulating inhibitory neurones. Signs of excitation followed the injection of tyramine and this may be a reflection of a predominantly stimulant action.

The depressant effect of intravenously injected tyramine on the polysynaptic flexor and crossed-extensor reflexes appeared similar to the effect of intravenously injected adrenaline (Schweitzer & Wright, 1937) and topically applied dopamine (McLennan, 1961) on the monosynaptic knee-jerk in the cat. By intravenous injection, dopamine was relatively very weakly active both in the cat (McLennan, 1961) and in the chick. However, it seems likely that the two closely related amines, tyramine and dopamine, may act at the same site.

The finding that strychnine was fully effective in the chick on the first day after hatching suggests that inhibitory mechanisms in the CNS are already well developed at this early age. The chick therefore differs from the kitten in which inhibitory processes develop slowly and in which strychnine is without effect up to 3 to 6 days after birth (Malcolm, 1955). The fact that strychnine was most potent in the youngest chicks and gradually became less so with increase in age, may reflect the gradual development of the blood-brain barrier. Even in the youngest chicks, however, strychnine was less potent on a body weight basis than it is in the adult cat probably because inhibitory mechanisms in the fowl never become as well developed as in the mammal.

The weak and transient effects of huge doses of tyramine on the blood pressure, particularly in the cat, seemed to be due to a self-blocking action since the pressor effect of subsequent doses of tyramine and noradrenaline were abolished. We did not test the effect of non-sympathomimetic pressor agents, so we cannot say whether the blocking action of large doses of tyramine was due to a specific α-receptor blocking action or to a general impairment of the reactivity of the cardiovascular system. It is possible that this self-blocking action of tyramine contributes to the development of tachyphylaxis to this amine when a series of smaller doses is injected. Spriggs (1964) has recently reached a similar conclusion from experiments on rats.

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