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EFFECT OF PENTOBARBITAL ON THE NORADRENALINE RELEASE INDUCED BY DRUGS AND FIELD ELECTRICAL STIMULATION FROM CEREBRAL AND FEMORAL ARTERIES OF THE CAT

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Abstract—The present studies showed that field electrical stimulation, high potassium (K^+), tyramine and ionophore X537A induced tritium release from cerebral and femoral arteries of cat prelabelled with [^{3}H]noradrenaline. The secretion caused by K^+ or field stimulation was Ca^{2+}-dependent and was antagonized by high concentrations of pentobarbital (10^{-4} and 10^{-3} M), whereas that induced by the rest of the drugs was unchanged in the same situations. The noradrenaline uptake by these arteries was reduced by pentobarbital (10^{-3} M and 10^{-4} M). These results suggest that this barbiturate interferes with Ca^{2+} entry to the adrenergic nerve endings, and therefore antagonizes the noradrenaline release by Ca^{2+}-dependent processes (exocytosis).

It is known that the barbiturates are clinically used to produce central depression ranging from sedation to coma. Concerning this, these agents have been reported to depress the Na^+ conductance [1, 2], Ca^{2+} permability of cell membrane [3–7] and Ca^{2+} uptake activities of microsomal [8] and mitochondrial fractions [9]. They also interfere with transmission in sympathetic ganglia by blocking the Ca^{2+} influx, at least partially, and the subsequent transmitter release in presynaptic endings [3]. Barbiturates may depress the transmitter release or synaptic excitation thus reducing the synaptic transmission [10]. Pentobarbital inhibits the noradrenaline secretion by nerve stimulation or depolarization [11–14] and the catecholamine uptake in mouse brain synaptosomes [15].

Recently, high doses of barbiturates have been used to treat several neurological conditions, including intracranial hypertension [16, 17] and the management of cerebral vasospasm [18]. However, the mechanisms by which they produce these beneficial effects are not known as some authors suppose that barbiturates produce vasoconstriction of the cerebrovascular bed [18, 19], whereas others have observed that they cause vasodilation in isolated cerebral arteries of goat and man [6, 7].

In spite of these studies, little is known about the effect of barbiturates on the noradrenaline release induced by different methods from cerebral arteries. Therefore the objective of the present study was to analyse the effect of pentobarbital on the noradrenaline secretion in cat brain arteries, induced by field electrical stimulation, potassium (K^+), tyramine and ionophore X537A, whose mechanisms of transmitter release have been analysed in peripheral arteries. Thus, field stimulation and K^+ induced noradrenaline release from adrenergic nerves occur by a Ca^{2+}-dependent process [20–23], whereas the release of noradrenaline by tyramine and ionophore is independent of extracellular Ca^{2+} [24–27]. The effect of pentobarbital on the noradrenaline uptake in these vessels was also analysed. The same experiments were also carried out in cat femoral arteries for comparative purposes.

MATERIALS AND METHODS

Cats of either sex, 1.5–4 kg body wt, were anaesthetized with 35 mg/kg of sodium pentobarbital and killed by bleeding. The brain and femoral arteries were carefully removed. The arteries of the circle of Willis with their branches and femoral arteries were cleaned to remove traces of blood. Each type of vessel of various animals was divided in cylindrical segments 4–6 mm in length, which were pooled and then separated in groups, each one of similar weight. Each group was placed in a cylindrical nylon net. After a 15 min equilibration period in Krebs–Henseleit solution (KHS) at 37°, the tissues were immersed in 5 ml of a (±)[^{3}H]noradrenaline solution (2×10^{-7} M, sp. act. 13 Ci/mmole) for 1 hr. The solution was continuously aerated with a 95% O_2–5% CO_2 mixture and kept at 37°. After this incubation period the arteries were transferred into a superfusion chamber at 37°, which contained platinum electrodes for field electrical stimulation (16 Hz, 0.5 msec, 80 V, for 1 min), and through which a prewarmed KHS was allowed to perfuse at a constant flow rate of 0.5 ml/min by means of a perfusion pump. A stream of O_2 with 5% CO_2 was passed at the same time through the chamber for 106 min by which time the spontaneous tritium outflow had reached a steady level. Afterwards two or four samples of the effluent were collected at 3 min intervals in order to determine the basal level of tritium release before electrical stimulation or drug addition.

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The radioactivity secretion evoked by field stimulation was collected during two intervals of 1.5 min (for better observation of the peak of this secretion) followed by two others of 3 min sampling period with the purpose of recovering the basal level of radioactivity. In order to analyse the tritium release caused by drugs (10^{-4} M tyramine, 10^{-5} M ionophore X537A and 120 mM K^+), KHS, which contained these drug concentrations, was passed through the chamber for 12 min, collecting the effluent every 3 min, followed by five others of 3 min sampling period for recovering the basal level of radioactivity. Aliquots (0.5 ml) of the samples were added to vials containing 2 ml of Bray's solution and the radioactivity measured in a Nuclear Chicago scintillation counter, model Isocap 300.

The basal tritium release before drug addition (pre-drug efflux) or electrical stimulation (pre-stimulation efflux) was given the value 1, and this was expressed as cpm/mg/min. The release caused by field stimulation or by drugs was calculated with respect to this value.

To analyse the influence of pentobarbital (10^{-4} M or 10^{-3} M) on [^{3}H]noradrenaline uptake in cerebral and femoral arteries, the barbiturate was added to the bath 10 min before and during the incubation period with the amine. Afterwards the arteries were washed with KHS for 30 min, and weighed and digested with 0.5 ml soluene at 55° overnight. The tissue was then measured for radioactivity by adding 2 ml of Bray's solution, and was expressed in cpm/mg.

The composition of the KHS was (mM): NaCl, 115; $CaCl_2$, 2.5; KCl, 4.6; KH_2PO_4, 1.2; $MgSO_4.7H_2O$, 1.2; $NaHCO_3$, 25; glucose, 11.1; and disodium salt of ethylenediaminetetraacetic acid (Na_2EDTA), 0.03. When pentobarbital (10^{-4} or 10^{-3} M) was used in order to study its influence in the tritium release it was added to the bath 10 min before and during electrical stimulation or administration of the drugs. With the purpose of observing the effect of extracellular Ca^{2+} in the tritium secretion, the arteries were immersed in OCa^{2+}-KHS after 80 min of washing until the end of the experiment. The composition of OCa^{2+}-KHS was equal to KHS except that $CaCl_2$ was omitted and 1 mM EGTA was added to complex with the contaminating Ca^{2+}. When K^+ was used the NaCl was removed from KHS in order to maintain the osmolarity.

Tyramine was prepared as stock solutions in physiological saline containing 0.01% (w/v) ascorbic acid and kept frozen (−20°). X537A was dissolved

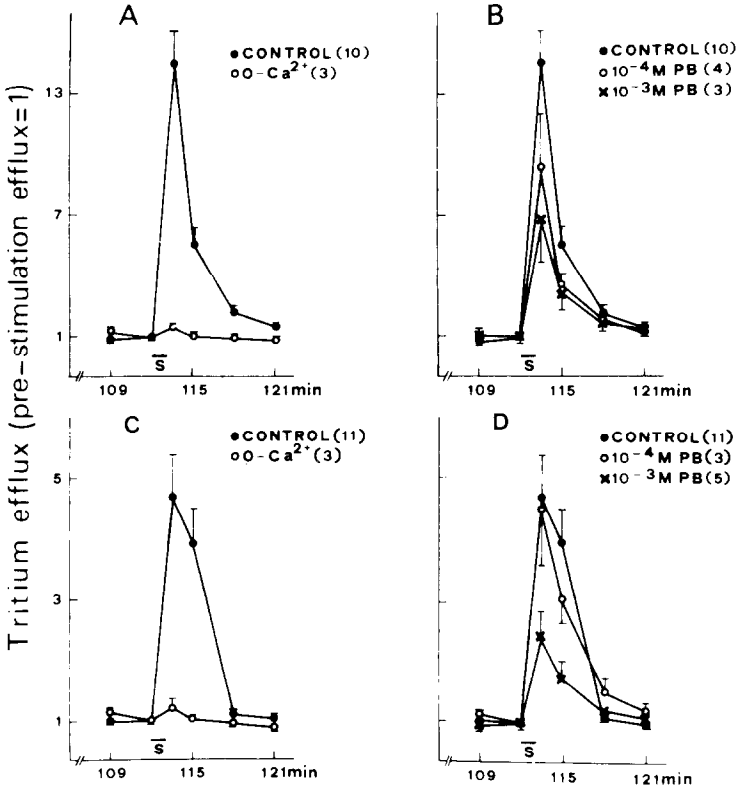

Fig. 1. The effect of pentobarbital (PB, 10 min preincubations) and the removal of extracellular Ca^{2+} from the medium on the tritium efflux evoked by field electrical stimulation (S) in cerebral (A, B) and femoral (C, D) arteries of cat. The vessels were previously loaded with [^{3}H]NA (2×10^{-7} M) and thoroughly washed during a 109 min period before the initiation of sample collection. Each point represents the tritium efflux during a period of 1 min (0.5 ml of superfusion fluid). The basal level was taken as 1 in all the treatments and the release caused by S was calculated with respect to this value. The number of experiments are shown in parentheses. Vertical bars represent S.E.M. The horizontal line indicates the time of exposure to S.

in ethanol and stored at −20°; the final concentration of ethanol in KHS was always 0.1%, which did not alter the tritium release induced by this drug [28].

The drugs used were: tyramine hydrochloride, Sigma Chemical Co. (London, U.K.); potassium chloride, Merck (Darmstadt, West Germany); ±[^{3}H]noradrenaline hydrochloride, Radiochemical Centre (Amersham, U.K.); ionophore X537A (Ro2-2985, Lasalocid, Hoffman-La Roche); sodium pentobarbital, Abbot (North Chicago, IL); and Soluene-100, Packard (Caversham, U.K.).

Results were expressed as means ± S.E. Deviations from the mean were statistically analysed by the Student's *t*-test. A probability value of less than 5% was considered significant.

RESULTS

Field electrical stimulation of cerebral and femoral arteries or cat induced a marked tritium release, which was abolished in Ca^{2+} free medium ($P < 0.01$, Fig. 1). The presence of pentobarbital (10^{-4} M) in the KHS did not cause a significant alteration in the secretion of radioactivity, but 10^{-3} M pentobarbital produced a significant decrease ($P < 0.05$, Fig. 1).

Exposure of these arteries to K^+ (120 mM) elicited tritium release, which was practically abolished by 10^{-4} M pentobarbital in femoral arteries ($P < 0.005$, Fig. 2) or significantly reduced ($P < 0.05$) or annihilated ($P < 0.005$) by 10^{-4} M or 10^{-3} M pentobarbital, respectively, in brain arteries (Fig. 2). The removal of Ca^{2+} from the medium markedly decreased the tritium secretion evoked by K^+ from these vessels ($P < 0.005$, Fig. 2).

Tyramine (10^{-4} M) and ionophore X537A (10^{-5} M) induced radioactivity secretion from cerebral and femoral arteries (Fig. 3) which was unmodified by pentobarbital (10^{-3} M) and by the omission of Ca^{2+} from the KHS.

The basal level of tritium release, pre-drug efflux or prestimulation efflux, was 25 ± 4 cpm/mg/min ($n = 10$) in cerebral arteries, and 16 ± 4 cpm/mg/min ($n = 11$) in femoral ones. These values were not significantly changed with pentobarbital (10^{-4} or 10^{-3} M) or by the removal of Ca^{2+} from the medium.

The tritium release evoked by field stimulation and by the three drugs was higher in cerebral arteries than in femoral ones, which accords with the greater noradrenaline content in the brain (1.3 ± 0.2 μg/g) [28] than femoral arteries (0.19 ± 0.22 μg/g, unpublished results).

The noradrenaline uptake (cpm/mg) by cerebral (3.420 ± 269, $n = 6$) and femoral (839 ± 45, $n = 6$) arteries was significantly reduced by: (a) 10^{-4} M pentobarbital (1596 ± 260, $n = 5$, $P < 0.02$) and (617 ± 7, $n = 4$, $P < 0.05$), respectively, and (b) 10^{-3} M

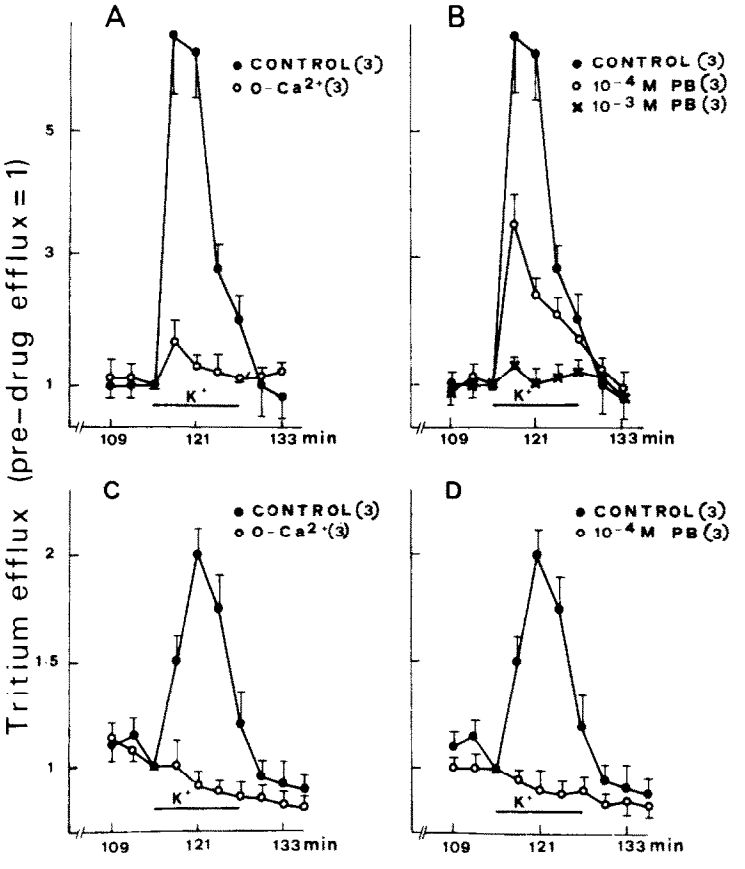

Fig. 2. The effect of pentobarbital (PB, 10 min preincubations) and the removal of extracellular Ca^{2+} from the medium on the tritium efflux evoked by K^+ (120 mM) in cerebral (A, B) and femoral (C, D) arteries of cat. Experimental design and the symbols as in Fig. 1.

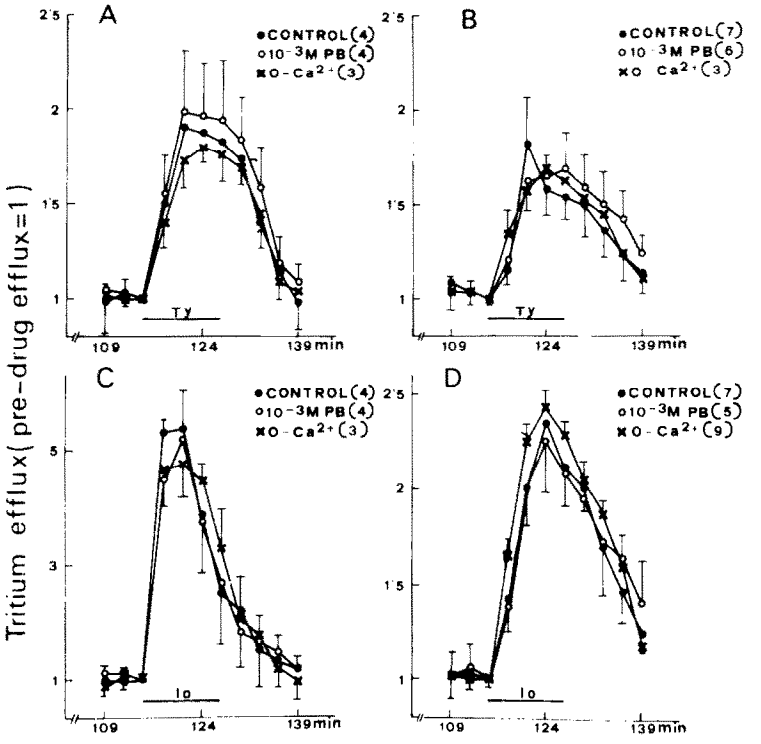

Fig. 3. The effect of pentobarbital (PB, 10 min preincubations) and the removal of extracellular Ca^{2+} from the medium on the tritium release evoked by tyramine (Ty, 10^{-4} M) and ionophore X537A (Io, 10^{-5} M) from cerebral (A, C) and femoral (B, D) arteries of cat. Experimental design and the symbols as in Fig. 1.

pentobarbital (897 ± 77, $n = 4$, $P < 0.001$) and (422 ± 23, $n = 3$, $P < 0.001$), respectively.

The effect of the drug was reversible. Fifteen to twenty min after removing pentobarbital, the arteries exhibit a normal response to loading and efflux.

DISCUSSION

The present experiments show that field electrical stimulation, high K^+ concentrations, tyramine and ionophore X537A induced tritium release from cerebral and femoral arteries of cat, whose endogenous noradrenaline stores had previously been labelled with this tritiated amine. The secretion caused by field stimulation and K^+ was Ca^{2+}-dependent, while that evoked by tyramine and ionophore was independent of extracellular Ca^{2+}. Pentobarbital reduced in a dose-dependent way the tritium release caused by K^+ and electrical stimulation, but unmodified that elicited by tyramine and ionophore. All these methods induced a greater radioactivity secretion in cerebral arteries than in femoral ones, which agrees with the fact that the brain vessels have a higher noradrenaline content than femoral ones.

It has been reported that K^+ and electrical stimulation produces a depolarization of adrenergic nerve terminals, which involves an increase in the Ca^{2+} influx from extracellular space augmenting the amount of Ca^{2+} free in the cell, and causing a noradrenaline secretion by exocytosis [20–22, 28]. In both cases the Ca^{2+} ions are an absolute requirement for the noradrenaline secretion. However, noradrenaline release elicited by tyramine and ionophore is by a Ca^{2+}-independent process [22–25, 27–29]. It is probable that both drugs could release the amine directly from the vesicles in the cytosol from which the neurotransmitter could then diffuse into the extracellular space. This hypothesis is also supported by the fact that these drugs do not produce a simultaneous release of noradrenaline and dopamine beta-hydroxylase [23–25].

The results obtained in the present study about the dependence or not of extracellular Ca^{2+} on the noradrenaline release, evoked by K^+, field stimulation, tyramine and ionophore, agree with those reported for other adrenergic innervated tissues. The fact that only the secretions induced by depolarization and subsequent Ca^{2+} influx into adrenergic nerve endings (K^+ and field stimulation) were inhibited by high concentrations of pentobarbital suggests that this barbiturate reduces the Ca^{2+} permeability of cell membrane. This hypothesis agrees with the fact that the concentration of the barbiturate used decreases the Ca^{2+} uptake by depolarized nerves [3, 4] and vascular tissues [5]. It is interesting to note that K^+-induced tritium release was more sensitive to pentobarbital (10^{-4} M significantly reduced this secretion) than to field stimulation. This difference could be due to the fact that K^+ depolarizes the membrane opening specific Ca^{2+} channels (essentially those that are potential sensitive) and extracellular Ca^{2+} passes through them,

whereas in the field stimulation, other channels (Na^+-selective channels) also participate to introduce Ca^{2+} [21], and probably are less affected by pentobarbital than those of K^+.

The mechanism by which the barbiturate interferes with Ca^{2+} entry to the cell is not exactly known, but it has been reported that it can produce an alteration of proteins of the cell membrane causing its expansion and fluidization [2], and in this way they block the Ca^{2+} channels. The interference of anaesthetic doses of pentobarbital (about 2×10^{-4} M) with release by K^+ or electrical stimulation is similar to the effect obtained in the heart of different animals [11, 13]. The fact that the noradrenaline secretion caused by tyramine and ionophore was Ca^{2+}-independent and was unaffected by pentobarbital additionally supported the hypothesis that barbiturates produce a fairly specific change in cell membrane of perivascular adrenergic nerves, because if this was unspecific it would decrease all secretions. They probably alter the specific conformational changes that occur in the membrane when it is depolarized, thus interfering with the Ca^{2+} entry.

Pentobarbital reduced in a dose-dependent manner the noradrenaline uptake in both types of arteries, which corroborates experiments on mouse brain synaptosomes [15, 30] where this barbiturate decreased the catecholamine uptake. However, in rabbit heart this transport process is not affected by this drug [11]. All these facts suggest that pentobarbital reduced the uptake of neurotransmitter in the adrenergic nerves of cerebral and femoral arteries, the mechanism of which is at present unknown.

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